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CONTENTS

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Juvenile Hormone III Hydrolyzing Activity in the Last Instar Larval Haemolymph of Tassar Silkworm *Antheraea mylitta* Drury.

R. V. Satyanarayana Rao and K. N. Mehrotra
Division of Entomology, Indian Agricultural Research Institute
New Delhi 110 012

Received on January 4, 1994.

Abstract: Last instar larval haemolymph of *Antheraea mylitta* D. showed the presence of both a-naphthyl acetate esterase (a-NacE) and juvenile hormone III esterase (JH III E). Both the enzymes had very high specific activity against their respective substrates indicating that apart from JH III E, a-NacE is also very essential at the time of metamorphosis of the larva into the pupal stage. Kinetics of substrate hydrolysis showed that a-NacE followed normal Michaelis Kinetics. JH III hydrolyzing enzyme had very high K_m, v_{max} and K values indicating the involvement of multi enzyme systems and other factors, Inhibition studies with paraoxon, DFP and a-Nac indicated that a-Nac esteraseand JH III E are two distinctly different enzymes and the JH III E is a DFP sensitive esterase.

Key words: a- naphthyl acetate esterase, juvenile hormone III esterase, *Antheraea mylitta*, kinetics, substrate hydrolysis, inhibition).

INTRODUCTION

Carboxyl ester hydrolases or Carboxyl esterases (CEs; EC 3.1.1.1) are heterogeneous group of enzymes with broad and over lapping properties (Mehrotra & Phokel, 1987). Juvenile hormone (JH), an acyclic sesquiterpenoid epoxide, plays an important role in metamorphosis and in regulating reproductive maturation in insects (Koeppe *et al.*, 1985; Riddiford, 1985). CE^S are known to play an important role in the metabolism of Juvenile hormones (JHs) in insects (Hammock, 1985; Role & Venkatesh, 1990). There are conflicting opinions on the enzymes metabolizing α and β-naphthyl acetates (Nacs) and JH and the DFP sensitivity or insensitivity of the JH esterases (Raushenback *et al.*, 1987). The present study reports the presence of esterases specific to α Nac and JH III from the presence of esterases specific to α Nac and JH III from the last instar larval haemolymph of tassar silkworm *Antheraea mylitta* D., their kinetic parameters of substrate hydrolysis and of inhibition by paraoxon, DFP, carbaryl, α-Nac and ortho-nitrophenyl acetate (*o* -NPA).

MATERIALS AND METHODS

Insect culture: Insects used in this study were last instar larvae of tassar silkworm *Antheraea mylitta* Drury obtained from Central Tassar Research and Training Institute, Ranchi, Bihar, India.

Preparation of haemolymph enzyme extract:

Haemolymph from the last instar silk worm larvae was collected in culture tubes which were kept at O°C in a freezing ice mixture and was then diluted by the addition of Tris-HCI buffer (0.1M, pH 7.4) containing 10 mg of phenyl thiourea (PTU) per 100 ml. The diluted haemolyph was centrifuged at 10000 g at 0-4°C for 15 minutes to remove haemocytes and other cellular inclusions. The supernatant was divided into aliquots of about 0.5 ml and stored at 15°C till further use.

Determination of enzymatic activity: α-Nac esterase activity was estimated spectrophotometrically by following the increases in the absorbance at 500 nm using α-Nac as substrate in Tris-HC1 bufffer (0.1M, pH 7.4) at 30°C (Chambers, 1976). Protein content was 752 µg/ml reaction mixture. Eight substrate concen-

trations from 0.1164 mM to 1.7453 mM ml^{-1} reaction mixture were used. The amount of α-Nac hydrolyzed during the reaction was estimated by using a molar extinction coefficient of 13.944 x 10^3 for the complex of α-naphthol and Fast blue RR salt.

The activity was measured according to Hammock & Sparks (1977) without adding ammonium hydroxide. The assay was carried out in 10 x 75 mm glass tubes at 30°C and the protein content varied from 5.6μg to 45.1 μg in the total reaction mixture. Control experiments with only buffer and heat treated haemolymph were also run simultaneously for corrections in partitioning of JH/JH acid. Rate of substrate hydrolysis was estimated at different time intervals.

Enzyme inhibition studies: Inhibition studies of JH III hydrolyzing activity were carried out using carbaryl, DFP, *o*-Nac and paraoxon as inhibitors. The stock solutions of inhibitors were prepared in methanol and stored at-15°C. Further dilutions of stock solutions were effected with methanol as and when required. The range of concentrations tried were 1 x 10^{-4} to 1 x 10^{-7} M of paraoxon, 2.5 x 10^{-4} to 1 x 10^{-7} M of paraoxon, 2.5 x 10^{-4} to 1 x 10^{-7} M of DFP, 9.09 x 10^{-3} to 2.2725 x 10^{-3} M of carbaryl, 8.2751 x 10^{-3} to 1.03 x 10^{-3} M of α-Nac and 8.0763 x 10^{-2} to 1.607 x 10^{-3} M of *o*-NPA. Care was taken that methanol content was not more than 1% in the final reaction mixture. The enzyme and the inhibitors were incubated for 10 to 15 minutes at 30°C and the substrate (labeled JH III mixed with appropriate quantity of unlabeled JH III) was then added and the assay carried out as described earlier.

Protein content in the incubates was determined by the method described by Lowry *et al.*, (1951) at 610 nm using bovine serum albumin (BSA) as a standard.

All spectrophotometric estimations were done using a double beam spectrophotometer Uvikon-810, Kontron Ltd. For radioactivity counting by liquid scintillation method, a Kontron liquid scintillation counter Beta natic -1 was used. The counts were corrected for quenching by sample channel ratio and the radioactivity was expressed as disintegrations per minute.

Statistical calculations: Kinetic parameters of α-Nac esterase were estimated by a parametrically robust method (Wilkinson, 1961). Since the other methods of estimation were giving variable values, kinetic parameters of JH III metabolizing enzyme(s) in the reaction mixture were estimated by the direct linear plot method of Cornish-Bowden & Eisenthal (1978).

Calculations of kinetic parameters was done using a programmable calculator (Hindustan Micro-2200) with extended memory. For estimating the half-life of the ^3H JH III in the reaction mixture, the following equation was used.

$$t_{0.5} = 0.693/K$$

where K is the pseudo first order rate constant for the over all reaction (Segel, 1976). I_{50}, the concentration of the inhibitor required to bring about a 50% reduction in the activity of the enzyme was calculated by plotting probit percent inhibition against the log concentration of the inhibitor.

Results and Discussion

α-Nac hydrolysis by larval haemolymph: Carboxyl esterases hydrolyzing α-Nac (α-NacEs) were reported from many insects and esterases hydrolyzing *o*-NPA were reported from the haemolymph and fat body of lst larval instar of *A. mylitta*[1]. From the experiments it was observed that the esterase was present in the last instar larval haemolymph of *A. mylitta* also. The specific activity of the enzyme ranged from 39.0 to 54.0 nmoles min^{-1}/mg protein (1.761 to 2.430 μ moles min^{-1} $l_{/ml}$ haemolymph). The kinetic parameters of the α -NacE estimated by statistically robust parametric method indicated a K_m of 4.63 x 10^{-2} ± 1.33 x 10^{-2} mM, V_{max} of 5.28 x 10^{-3}± 1.45 x 10^{-4} nmoles/g protein/min. and K (V^{max}/K^M) of 1.14 x 10^{-1}/min.

JH III hydrolysis by larval haemolymph:

From the results on the hydrolysis of JH III it can be concluded that the larval haemolymph of *A. mylitta* has JH III hydrolyzing activity.

Table 1. Kinetic parameters of *A. mylitta* haemolymph enzyme calculated by Cornish-Bowden and Eisenthal (1978) method using JH III as substrate.

Sl. no.	Protein content µg	Apparent K_m* (mM)	Apparant V_{max}* nmoles/g protein/min.	k* (V_{max}/K_m) /min.
1	45.1(1)	0.02 (0.001 - 0.03)	0.07 (0.003 - 0.12)	0.1679 (0.069- 0.1718)
2	45.1(2)	0.62 (0.10 -3.90)	0.91 (0.15 - 5.68)	0.0658 (0.0656- 0.0661)
3	22.6	2.03 (0.48 - 88.76)	7.02 (1.64 -306.49)	0.078 (0.0779 -0.0781)
4	11.3	4.58 (1.21 -145.26)	17.12 (4.5 - 542.92)	0.0422(0.421 - 0.423)
5	5.6	0.76 (0.22 -5.39)	2.70 (0.78 - 19.14)	0.0201(0.0199 - 0.0202)

* Median values for a group of 16 values, with the ranges given in the parentheses.

A closer scrutiny of the rate of hydrolysis at the highest protein concentration in the reaction mixture revealed two distinct hydrolysis curves, the first curve having a greater initial hydrolysis rate than the second (Fig. 1) suggesting that there may be two enzymes working on JH III. The analysis of JH III hydrolysis at this protein concentration was carried out by following a curve peeling method of Van Liew (1967). As a result of this peeling method, the kinetic parameters like K_m, V_{max}, K and $t_{0.5}$ of JH III in this protein concentration were estimated separately for the two peelings.

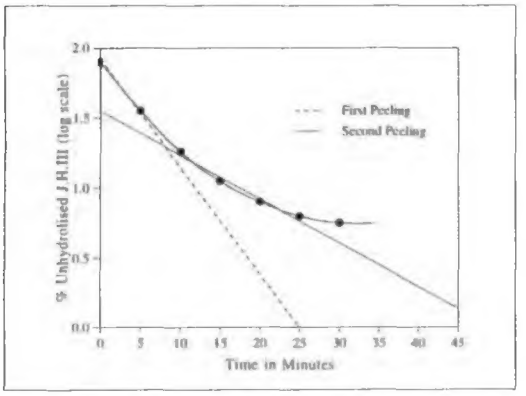

Fig. 1. Van Liew's (1967) curve peeling method for JH III hydrolysis in *A. mylitta* haemolymph enzyme. Reaction mixture contained 5 µl of substrate solution (250 pmoles of JH III) and 100 µl of enzyme. The reaction time varied from 5 minutes to 30 minutes. Protein content in the reaction mixture was 45.1 µg.

Estimation of kinetic parameters of JH III hydrolysing enzyme(s) by the direct linear plot method of Cornish - Bowden and Eisenthal (1978) using 1/v and K_m/v as the primary parameters of Michaelis - Menten equation yielded the K_m, V_{max} and L (V_{max}/K_m) values in the negative as well as positive range. However, Based on their own explanation all the negative values were taken as positive values by treating them as "beyond infinity" rather than as "below zero".

The kinetic parameters of JH III hydrolysis as shown in table 1 show the median value of apparent K_m, V_{max} and K which varied according to the protein concentration in the reaction mixture. The apparent kinetic parameters ranged from 0.02 to 4.58 mM for K_m; 0.066 to 17.12 moles/g protein/min for V_{max} and 0.1679 to 0.0201/min for K in the different protein concentrations indicating that it is a complex reaction. These values are found to be comparatively very high to the values available from different sources (1×10^{-7} to 40×10^{-7} M for K_m) in the literature (Hammock, 1985).

The possible reasons for this high values in the present study could be, (1) species specificity of the enzyme as found in the case of *Leptiontarsa decemlineata* (Kramer & de Kor, 1976a, b); (2) Binding protein(s) might have caused an elevation of the apparent K_m value; (3) Involvement of more than one enzyme from the crude enzyme systems hydrolysing JH in insects was reported in the literature (Hanzlik & Hammock, 1987; Woxniak *et al.*, 1987; Jesudason *et al.*, 1990; Brown *et al.*, 1977; Hammock *et al.*, 1977; Kramer & Childs, 1977; Peter *et al.*, 1979; Roe *et al.*, 1983 and

Lessman & Herman, 1984).

When compared to the K_m and V_{max} values, the K values (Table 1) were found to give a stable positive correlation with the protein concentration, with the highest protein concentration of 45.1 µg having the highest K value of 0.1679/min and the lowest protein concentration of 5.6 µg having the lowest K value of 0.0201/min.

Table 2. Half life of (^{3}H) JH III in *A. mylitta* haemolymph.

Sl. No.	Protein content µg	$t_{0.5}$ value (min)
1	45.1 (1)	3.75
2	45.1 (2)	10.53
3	22.6	8.89
4	11.3	16.42
5	5.6	34.61

* Substrate concentration in the reaction mixture was 2.39 x 10^{-6} M.

The half life ($t_{0.5}$) value of JH III in the reaction mixture (Table 2) revealed that it followed the normal enzyme kinetics with lowest $t_{0.5}$ value at the highest protein concentration and highest $t_{0.5}$ value at the lowest protein concentration in reaction mixture.

Enzyme inhibition studies: α-NacE: The inhibition studies of α-NacE carried out with paraoxon and DFP as inhibitors showed that the larval haemolymph α-NacE was not inhibited even at 3.3 x 10^{-5} M paraoxan pr 1.81 x 10^{-5} M DFP indicating that these are specific esterases when compared to most of the common α-NacEs found in different species. Similar observations were made in α-NacE (mol. wt. 1,40,000) from *G. mellonella* haemolymph which was inhibited to only 57% at a concentration of 2 x 10^{-4} M DFP (Rudinicka *et al.*, 1979).

JH III hydrolyxing enzyme: Inhibition studies carried out with JH III hydrolyzing enzyme from larval haemolymph of A. *mylitta* using paraoxon, DFP, carbaryl, α-Nac and o-NPA as inhibitors, as shown in table 3 revealed the following points.

The I_{50} value of paraoxon on JH III hydrolyzing enzyme was 3.71 x 10^{-8} M. This indicates that the enxyme is an esterase and not JH epoxide hydrase as JH epoxide hydrases are known to be highly resistant to the inhibition by paraoxon (Hammock *et al.*, 1977; Yu & Terriere, 1978).

The enzyme was found to be sensitive to DFP with an I_{50} of 1.07 x 10^{-5} M while α-NacE from the same source was found to be refractory to both paraoxon and DFP.

Insects treated with organophosphates were reported to have hyper-hormonal condition (Samaranayaka-Ramasamy, 1978). Perhaps this might be due to the inhibition of JH esterases by these insecticides as the JHE I_{50} values are comparable to that of acetylcholineesterase which were the main target of the insecticides.

Carbaryl, α-Nac and o-NPA were found to be poor inhibitors of JH III hydrolyzing enzyme from the larval haemolymph of *A. mylitta*. The inability of both α-Nac, o-NPA in the inhibition of the enzyme competitively indicates that the enzyme is not the general carboxylesterase.

From the above it can be concluded that in *A. mylitta*. α-NacE whose natural substrate in the insect system is not known, is also needed at the time of metamorphosis of larva to pupa and JH III E and α-NacE are distinctly different enzymes. However, studies by Hanxlik *et al.*, (1989) on the gene encoding JHE from *Heliothis virescens* revealed amino acid sequences which had about 24% similarity to several other serine esterases, indicating that some amount of similarities exists between α-NAcE and JHE.

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ENTOMON 1995 VOL. 20 NO. 2: 7-11.

Changes in quantitative and qualitative patterns of Esterases in developing eggs of *Chrysochoris purpureus* Westw.

K. Shobha Rani and V. Lakshmipathi
Department of Zoology, Kakatiya University
Warangal 506 009

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Abstract: Changes in quantitative and qualitative patterns of esterases and proteins were observed in the developing eggs of *Chrysochoris purpureus*. The developing eggs were divided into four stages based on the appearence of the pigmented band. Newly hatched juvenile formed the fifth stage in the study. Five zones of esterases were observed in the developing eggs, of which only three were present in all the stages of development. One of the zones (zone- 3) is active from stage III-V.

Key words: *Chrysocoris purpureus*, Esterase, Electrophoresis, eggs.

INTRODUCTION

Although quite a good number of reports exist on esterase patterns in insect tissues (Arurkar and knowles 1968, Freyvogel *et al.* 1968, Laufer 1960). Only few studies were made on the patterns of esterases observed in the developing insect eggs (Salkeld 1965, Guss and Krysan 1972, Kai and Nishi 1976). Esterases were shown to play a lipolytic role in the developing eggs (Guss and Krysan 1972) and activation of some esterases induced morphogenesis in diapausing eggs (kai and Nishi 1976). Blastokinetic movements occurring in early embryogenesis of *Chrysocoris purpureus* were described by Swaminathan and Sriramulu (1975). The present paper described the changes in both quantitative and electrophoretic patterns of esterases at different stages of egg development i. e., from freshly laid eggs to newly hatched juveniles.

MATERIALS AND METHODS

The insects, *Chrysocoris purpureus* , collected from the fields around the Kakatiya University campus were maintained on their natural diet. The developing eggs were divided into five stages on the basis of appearence of pigmented band according to the method of Swaminathan and Sriramulu (1975).

Stage I	Freshly laid eggs.
Stage II (72 h)	Pigmented band appearing on the lateral side of the cephalic lobe.
Stage III (84-96 h)	Pigmented enlarged band forming a crescentic cap like structure.
Stage IV (102-120h embryo)	Orange coloured eggs with dorsal closure completed.
Stage V (8 days old embryo)	Freshly hatched juveniles.

Activity levels of esterases were estimated according to method of Van Aspereen (1959). The total assay mixture (6 ml) contained 5.0 ml of substrate in phosphate buffer (pH 7.5) and 1.0 ml of tissue extract. The reaction mixture was incubated at room temperature at 30°C for 20 minutes. The reaction was arrested by the addition of one ml of chromogen solution containing 2 parts of 1% solution of fast blue B and 5 parts of 5% sodium lauryl sulphate solution. The colour developed after the addition of fast blue B was read against the reagent blank at 600 mu in a colorimeter.

Electrophoresis: The homogenates (10%) of the embryos were prepared in distilled water and centrifuged at 2000 rpm for 10 minutes at room

temperature (30°C). The supernatants were mixed with equal volume of 20% sucrose solution containing bromophenol blue as a tracking dye. An aliquot of 0.1 ml of this solution was used for electrophoresis. A discontinuous buffer system with Tris (0.025 M) and glycine (0.192 M), pH 8.3 as the gel buffer and Tris (0.5 M) EDTA (0.016 M) and borate (0.65 M) buffer diluted 1: 9 with distilled water (pH 8.3) as electrode buffer was used for electrophoresis. A constant current of 20mA was applied for the first 15 minutes following which the current was raised to 30mA and the electrophoresis was terminated when the tracking dye migrated to a distance of 12 cm from the origin. Staining procedures for esterases and the methods of classification of the zones were described earlier by Lakshmipathi and Reddy (1989). The relative proportion of activity contributed by individual esterase zones was determined according to the procedure of Klebe (1975) which has been described earlier (Lakshmipathi & Sujatha 1991).

Results and Discussion

Changes in activity levels of esterases are presented in Table 1. Electrophoretic patterns of esterases of the developing eggs are shown in Fig. 1 and the details about the relative mobility, visual endpoints and classification of the zones are presented in Table 2.

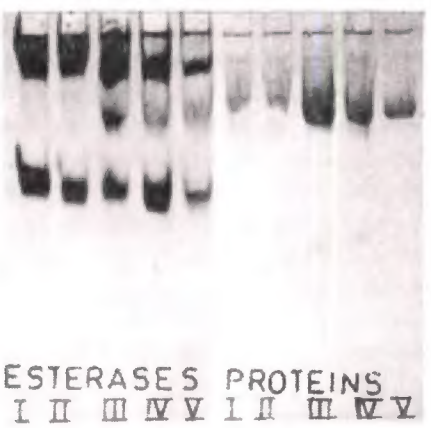

Fig. 1. Patterns of esterases and proteins found at different stages of development (I, II, III, IV and V are stages of embryos).

The results indicate that activity levels of esterases deplete marginally in embryos at stages III and IV but rise by about 1.6 folds in the newly hatched 1st instar nymphal stadium (stage-V). Electrophoretic studies (Table 2) indicate that there are five electrophoretically detectable zones in the embryos, out of which only three zones viz: zones 1,2 and 5 appear in all the five stages of development. Zone 3 appears in stage-II and increases in its activity during stages-III to V. Zone 4 is a weak band, which appeared only in stage-II. The visual end points of the zones indicate that the overall activity of the zones increased in stage-V. Classification of the zones, by using the inhibitors indicates that zones 1 & 2 are carboxyl esterases, zones 3,4 & 5 are ER esterases not effected by the inhibitors. Zones 1, 3 and 5 could hydrolyze the laurate ester. The protein patterns obtained on the second half of the gel of the same sample, indicates that three of the protein zones with Rm 20, 16 & 8 migrate close to esterase active zones. The protein band with Rm 20 is densely stained with coomassie brilliant blue and appears only during stages III-V. Protein band with Rm 16 depletes during these stages.

Table 1. Changes in activity levels of esterases. Data from 6 batches of eggs. µ moles of Naphthol formed/ egg/min (FC=Folds of change).

	Stae I	Stage II	Stage III	Stage IV	Stage V
Mean		3.55	3.13	2.90	5.35
SE	3.41	±0.135	±0.056	±0.052	±0.517
FC	±0.64	1.04	0.91	0.85	1.56
P		NS	<0.01	<0.001	<0.01

Esterases and Lipases are reported in several species of insect eggs (Agrell and Lundquist, 1973). Lipases are reported to be present extensively in yolk (Karlson, 1941). *In vivo*, the yolk lipids are thought to be protected from lipases by a membrane bound barrier (Krysan and Guss, 1973). Salkeld (1965) reported a number of esterase-active zones in the embryos of *Oncopeltus fasciatus*.. Eudy and Dobrogasz

Table 2. Relative mobility, activity with 1-naphthylacetate, visual end point and classification of esterase zones obtained on the zymogram at different stages of development

Zone	1	2	3	4	5
Stage No. Rm	38.3	35.8	20.8	16.6	8.3
Stage I					
Activity	+++(L)	+++			++(L)
Visual end point	16	64			32
Classification	CE	CE			CR
Stage II					
Activity	++(L)	+++	+	+	++(L)
Visual end point	32	64	4	8	32
Classification	CE	CE	ER	ER	ER
Stage III					
Activity	++(L)	+++	++(L)		++(L)
Visual end point	16	32	8		64
Classification	CE	CE	ER		ER
Stage IV					
Activity	++(L)	+++	++(L)		++(L)
Visual end point	16	128	16		128
Classification	CE	CE	ER		ER
Stage V					
Activity	++(L)	+++	++(L)		++(L)
Visual end point	8	64	8		128
Classification	CE	CE	ER		ER

Note:

Rm = Relative mobility is given as percent migration of the zone to that of tracking dye.

+++ = High activity; ++ = Moderate activity; + = Low activity; -L = in parenthesis indicates laurate ester hydrolyzing ability of the zone. Visual end point is the dilution at which the last visible band appeared on the zymogram. CE = Carboxyl esterases; ER = Esterases resistant to inhibitors.

(1970) reported an increase in esterase activity during the last 30% of embryonic development. Guss and Krysan (1972) demonstrated soluble esterases in the embryos of *Diabrotica* species. Two of the electrophoretically separated zones in these species were also shown to exhibit lipolytic activity and were shown to be sensitive to inhibition by paraoxon (Krysan and Guss, 1971). Kai and Hasegawa (1973) demonstrated that one of the esterases (esterase-A) is involved in the termination of diapause in silkworm. Carboxyl esterases present in the haemolymph of insects were implicated in the catabolism of juvenile hormone esters (Slade and Zibitt, 1972) and *in vitro* they were shown to hydrolyze the juvenile hormone esters. Juvenile hormone esterases (JHE) were shown to be active during larval development of *Manduca sexta* (Sanburg *et al.* 1975). One of them (JHE-I) was shown to be protected by a carrier protein and was found to be sensitive to inhibition by the organophosphate compound (DFP), while the other (JHE-II) was shown to be resistant to inhibitors. The latter enzyme appears only during larval pupal metamorphosis (Sanburg *et al.* 1975). Since juvenile hormones play a key role in the development of insects from embryos to adults (Schneiderman, 1972) and they are stored in the insect eggs during oocyte development (see Bownes, 1990), it can be suggested that esterases in insect eggs play the dual role of hydrolyzing the yolk lipids as

well as the hydrolysis of juvenile hormones, which facilitates morphogenic events in the developing embryos. The appearance of zone 3 at stage-II (germ band formation and elongation) and the increase in its activity during subsequent stages of development can be attributed to its role in morphogenesis. Like JHE II in *Manduca seta* (Sanburg *et al* . 1975), this zones is also not affected by the inhibitors. Esterases, which were resistant to inhibitors are shown to be active during the final stage of embryonic development of several insects and are believed to be associated with neuroblast formation in developing embryos (wigglesworth 1958, Salkeld 1961, 1964, Edwards and Gomez 1966, and Smith and Salkeld 1966). The laurate ester hydrolyzing ability of some of the esterase zones in the present investigation indicates their lipolytic function (Pearse 1972). Hydrolysis of yolk lipids, especially the neutral lipids, liberated glycerol which plays a role in hatching of embryos (Kinsella 1966).

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The Cuticular Lipids of the Larva of *Antheraea mylitta* Drury

P. P. Srivastava, N. D. Banerjee and K. Thangavelu*

Central Tasar Research & Training Institute, P. O. Piska Nagri, Ranchi - 835 303 Bihar, India

*National Silkworm Seed Project, Central Silk Board Complex, B. T. M. Layout, Hosur, Madivala, Bangalore - 560 068, India

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Abstract: The lipids extracted from cast skins (exuviae) of *Antheraea mylitta* Drury larvae, were separated by chromatography on florisil column. Each fraction was determined by gas chromatography, thin layer chromatography, infrared spectroscopy and mass spectrometry. Most of the cuticular lipids (62%) are made up of two straight chain saturated alcohols (n - triacontanol and n- octacosanol). Among the other components, hydrocarbons comprise 11% of cuticular lipids, free fatty acids 5%, esters 11% and phospholipids 1%.

The hydrocarbons are all saturated. The two principal hydrocarbons were identified as heptacosane and nonacosane and these form respectively 24% and 55% of the hydrocarbon fraction. The free fatty acids were found to be myristic, palmitic, stearic, oleic and linoleic acids. The same fatty acids are present in the ester fraction. Palmitic acid is the chief component in both the cases.

Key words: Cuticular lipids; *Antheraea mylitta*, column chromatography; Thin layer chromatography; Gas-liquid chromatography.

INTRODUCTION

The function of insect cuticular lipids in water conservation and protection from desiccation are well documented (Beament, 1961; Hadley, Machin & Quinlan, 1986). However, the chemical composition of cuticular lipids has been the subject of recent investigations. Henceforth, it has been studied in few insect species viz. *Anabrus simplex* (Baker *et al.*, (1960) *Periplanata americana* (Gilby, 1963) *Pteronarcys californica* (Arnold *et al.*, 1969), *Melanoplus packardii* (Blomquist *et al.*, 1972), *Manduca sexta* (Buckner *et al.*, 1984), *Anopheles maculatus* (Kittayapong *et al.*, 1990), and *Curculio caryae* (Espelie & Payne, 1991). Details on this aspect pertaining to sericigenous insects is lacking and hence this study was extended to tropical tasar silkworm species. *Antheraes mylitta* Drury which is wild in nature and has to encounter all the natural vagaries during its larval period. Since culticular lipid is shed with the cast skin (exuvium) at ecdysis (de Renobales & Blomquist, 1983), it is chosen as a suitable source of material.

MATERIALS AND METHOD

Lipids were extracted from the cast skins of *A. mylitta* larvae with warm chloroform methanol (2:1), following the procedure of Folch *et al.*, (Folch *et al.*, 1957). The solvents were removed under vaccum, and the lipids dissolved in warm hexane for application to a Florisil column (Floridin Co., Pittsburgh) prepared as described previously (Carroll, 1961). Column eluates were collected in 5 ml portions. After the removal of solvent under nitrogen and weighing, the lipid residues were checked for homogeneity by thin-layer chromatography using silica gel G.

The plates were developed with a hexane-diethl either-acetic acid (80:20:1) mixture, and the lipid constituents were detected by exposure to iodine vapour. Examination of the cuticular alcohols for unsaturation was made on thin layer plates impregnated with silver nitrate (Morris, 1963) and developed in hexane-diethl ether (4:1). Detection was accomplished by spraying with sulphuric acid and charring with heat. Appropriate standards were employed

with each chromatographic run.

The hydrocarbons were analysed by gas-liquid chromatography on a 6 ft all-glass column packed with 3% QF/1 on 60/80 Gas Pack/F, at 175°C. The methyl esters of fatty acids were analysed on a 9 ft all-glass column packed with 10% carbowax on 100/200 mesh acid and alkali washed celite, at 194°C. In both columns the carrier gas was nitrogen, at 15 psi, and the detector was a gas density balance.

Samples for mass spectrometry were run on an A. E. L. MS9 mass spectrometer, using a direct insertion probe at a source temperature of 200°C. Melting points were determined on the Kofler block.

Results

The Folch extraction procedure yielded 7.1% (by weight) lipid from the cast larval skins. The combined weights of the lipids from the column amounted to 90.7% recovery.

Alcohols: The alcohol fraction was shown to be a saturated alcohol by infra-red spectroscopy, thin-layer and gas-liquid chromatography and by the formation of several derivates. This fraction was 62% of the mass which was found to be free of contamination by TLC examination. Analysis by GLC of this alcoholic material revealed a minor and a major peak. Comparison of the retention times of this material with a log plot of known standard saturated straight chain alcohols indicated that the two peaks were in the order of increasing retention time, 2.0% C_{28} (n - octacosanol) and 98% C_{30} (n - triacontanol) m. p. 86.3°C (Francis *et al.*, 1937). Comparisons of the movement of these compounds with saturated and unsaturated alcohol standards on a silica gel thin-layer plate impregnated with silver nitrate also failed to show any unsaturated compound.

Hydrocarbons: The hydrocarbon fraction represented 11% of the cuticular lipid. Analysis of the hydrocarbons by gas-liquid chromatography showed the presence of four major and several minor components. These were identified by comparison with the log retention distances of known hydrocarbons and the relative proportions of the components were estimated on the basis of peak areas measured by triangulation. The results are depicted in table 1. There was no evidence of unsaturation in the i. r. spectra of the hydrocarbons. The principal hydrocarbons were heptacosane (C_{27}) and nonacosane (C_{29}), which formed 24 and 55% of the total hydrocarbons respectively.

Table 1. Gas chromatography of the hydrocarbon fraction

No. of carbon atoms	Log retention distance		% of fractions
	Standards	Unknowns	
C_{22}	0.92	-	-
C_{23}	1.11	-	-
C_{24}	-	1.23	7
C_{27}	-	1.55	24
C_{28}	1.75	-	-
C_{29}	-	1.90	55
C_{30}	-	2.12	10
C_{32}	2.34	-	

* Four per cent was C_{20} or C_{31}.

Esters: The lipid recovered in the ester fraction comprised approximately 11% of the cuticular lipid. It was a solid with a m. p. of 68°C, and the IR spectrum showed evidence of unsaturation. The saponifiable lipids were separated from the reaction mixture, methylated by treatment with an ethereal solution of diazomethane, and analysed by gas-liquid chromatography. The identity of the components was established by comparison of log retention distances with those of standard compounds, supported by co-injection with standard compounds (Table 2). The identification of the unsaturated components was checked by bromination of the mixture, which resulted in the disappearance of the corresponding chromatographic peaks. The relative proportions of the different components were assayed by the measurement of peak areas.

The principal fatty acids present were palmitic acid, stearic acid and oleic acid, Smaller amounts of linoleic acid and myristic acid were also present.

Table 2. Gas chromatography of the methyl esters of fatty acids from the ester and free fatty acid fractions.

Acid	No. of carbon atoms	Log retention distance		% of fraction	
		Standards	Unknowns	Ester	FFA
Lauric	C_{12}	1.37			
Myristic	C_{14}	1.68	1.66	1	1
Palmitic	C_{16}	1.98	1.98	66	41
Stearic	C_{18}	2.28	2.27	16	10
Oleic	C_{18}	2.30	2.30*	10	21
Linoleic	C_{18}	2.38	2.37*	7	27
Linolenic	C_{18}	2.46	-	-	

* Removed by bromination.

Free fatty acids: The free fatty acids obtained by column chromatography comprised 5% of the cuticular lipid. They were methylated and analysed in the same way as the fatty acids obtained from the ester fraction (Table 2). The same acids were present as in the ester fraction and again the principal component was palmitic acid.

Phospholipids: The material eluted from the column was dissolved in a small volume of methanol-chloroform (1:1), and the phospholipids precipitated by addition of 10 ml cold acetone. This comprised approximately 1% of the cuticular lipids.

Table 3. Summary of the composition of *A. mylitta* cuticular lipids.

Chemical class	% of lipid	Component class	% of class
Alcohols	62	C_{28}	2
		C_{30}	98
Hydrocarbons	11	C_{20}	2
		C_{24}	7
		C_{27}	24
		C_{29}	55
		C_{30}	10
		C_{31}	2
Esters$	11	C_{14}	1
		C_{16}	66
		C_{18}	16
		C_{18*}	10
		C_{18**}	7
Free fatty acids	5	C_{14}	1
		C_{16}	41
		C_{18}	10
		C_{18*}	21
		C_{18**}	27
Phospholipids	1	-	-
Not identified	1	-	-
Not recovered	9	-	-

* One double bond; ** Two double bonds; $ Refer to fatty acids only.

Other fractions: The quantities of material recovered in some fractions did not prove adequate to permit unequivocal identification.

The summary of the results showing the composition of *A. mylitta* cuticular lipids is shown in Table 3.

Discussion

The results obtained show that the cuticular lipids of *A. mylitta* larvae is a complex mixture in which straight chain saturated alcohols dominate over other fractions. In this way it differs from the cuticular lipids of many insects studied so far which have soft cuticle in which the proportion of hydrocarbons is high. Wax esters of long chain acids and alcohols have been found in the cuticles of only a few species, sometimes in considerable proportion of about 62% in a beetle (Baker, 1978). However, presence of high proportion of free alcohols (63%) in the cuticle of *A. mylitta* resembles with *Ceroplastes destructor* Newstead (Hackman, 1951) (27%) and *Samia cynthia ricini* (Bowers & Thompson, 1965) (92.6%).

The role of primary alcohols in reducing the water evaporation has been advocated by Gilby (Gilby, 1980), however, Bowers and Thompson (1965) have postulated that cuticular alcohols are not involved in water conservation but perform an entirely different service like restricting the growth of pathogenic fungi. The present study confirms the views of Bowers and Thompson. As *A. mylitta* is highly resistant to fungal diseases compared to other sericigenous insects (Sen *et al.*, 1970). Hence high cuticular content of free alcohol in *A. mylitta* is of high significance to the survival of *A. mylitta* larva against the onslaught of fungal diseases.

Further, the functional role of other cuticular components (Table 3) viz. hydrocarbons (11%), esters (11%), free fatty acids (5%), phospholipids (1%) is known to minimise the transpiration and thus to protect from dessication (Machin, 1980 and Toolson, 1980). However, the complex hydrocarbon mixture (C_{20} to C_{31}) recorded in the present study (Table 3) may be correlated with the logical conclusion of Lockey (1980). In this connection it was argued that the complex hydrocarbon mixtures of insects remain fluid or semi-fluid over a range of temperature and as well as contributing to the water impermiability of cuticular lipid and providing a fluid or semi-fluid matrix for polar constituents they could also, through their composition, control the viscosity of cuticular lipid. Viscosity could be an important factor in lipid secretion through the pore canal system of the cuticle and it could also be important in the spreading of lipid over the cuticular surface and in maintaining the integrity of the lipid layer.

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Use of Alternative Foods in the Rearing of Aphidophagous Ladybeetle, *Menochilus sexmaclatus* Fabr.

B. K. Agarwala & M. Sen Choudhuri
Department of Life Science, Tripura University
Agartala 799 004, India

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Abstract: Aphidophagous ladybeetle, *Menochilus sexmaculatus* developed and reproduced in captivity when offered alternative foods like drone honeybee powder, ant eggs or a combination of ant eggs and aphids (2:1). However, female ladybeetle showed longer pre-reproductive delay and laid less number of viable eggs when compared to those females fed on living aphids. Among the alternative foods, adults fed on drone honeybee powder oviposited more eggs and adults lived longer than those fed on ant eggs or a combination of ant eggs and aphids.

Key words: Rearing, alternative foods, ladybeetle, *Menochilus sexmaculatus*.

INTRODUCTION

Aphidophagous ladybeetles are important agents in the natural control of aphids (Hodek, 1973). Some of the potential ladybeetle species are being extensively studied in respect of biological and ecological properties prior to their field realase (Smirnoff, 1958; Smith, 1965; Nijima *et al.*, 1986). *Menochilus sexmaculatus* (Fabricius) is one such species. This ladybeetle species is a common predator of aphid and also scale insects in Far East and South East Asia (Hukusima & Kouyama, 1974). In view of common occurrence of this ladybeetle species in association with a wide range of aphid species (Agarwala & Ghosh, 1988). attempts are being made in recent times to augment the rearing of this ladybeetle on alternative foods in order to exploit its biocontrol potential in the field (Patnaik & Sahu, 1980; Niijima *et al.*, 1986). In our efforts to continue the search for suitable alternative food in the rearing of *M. sexmaculatus*, eggs of an ant species, *Oecophylla smaragdina* (Fabricius), drone honeybee powder made from *Apis mellifera* and soyabean food were tried in captivity. The results obtained are presented and discussed in this paper.

MATERIALS AND METHODS

Laboratory culture: Individual egg batch, larva or adult of *M. sexmaculatus* (Coleoptera: Coccinellidae) was obtained from the stock culture being maintained in the laboratory at ambient temperature by offering living aphids, *Aphis craccivora* koch. A batch of eggs, a larva or an adult was individually placed in a 5 cm diameter petridish. Adequate quantity of food by weight, aphid or alternative food, was provided on a piece of tissue paper to each of the experimental larva or adult. The food was replaced by fresh supply at an interval of 24 hr when observation was recorded. A piece of filter paper soaked with water was used as a source of moisture in the petridish. Experiments were performed at 20°C and 12 hr light: dark photoperiod in an environment chamber.

***Preparation of alternative food*:**

1. *Ant eggs* (AE) Fresh supplies of eggs of *O. smargdina*, commonly available in the local market as fish-bait, were periodically procured and stored in a deep freeze at - 5°C. Eggs of ants, about 3-5 mm long, were used as such in the experiment.

ii. *Ant eggs + Aphids (AE+A)* Ant eggs, as aforesaid, were crushed in a porcelin bowl and

mixed with fresh aphids (*A. craccivora*) in 2: 1 proportion by fresh weight. The mixed food was heated at 50°C for 30 minutes to remove excess water and then used in small flakes.

iii. *Drone Honeybee powder (DHBP)*: A sample quantity (250 g) of this food was obtained from the Institute of Honeybee Research, Tamagawa University, Japan for the present study. This food was prepared following the method described in detail by Matsuka & Niijima (1985).

iv. *Soyabeen Food (SF)*: Each 100 g of this food was made from the following constituents - soyabean powder (commercial) 23.24 g, natural honey 7.78g, agar 7.78 g, ascorbic acid 1.94 g, vitamin B- complex 0.78 g prepared in suitable quantity of distilled water.

***Laboratory experiments*:**

1. *Development:* First instar larva, within 12 hr of its emergence. (n=10, each for different food) was weighed in an electronic balance kept in a 5 cm diameter petridish. It was offered alternative food or living aphid in the following order: I instar - 1 mg, II instar - 4 mg, III instar - 10 mg, IV instar - 15 mg, pupa - none. The food was replaced by fresh supply at 24-hr interval. Observations were made at 12 hr interval in respect of duration of development of different instar larvae, pupa and emergence of the adult. Mortality, if any, in the experiment was recorded. A fully grown larva preparing for pupation was weighed again. Adults, within 12-hr of emergence, were sexed under the microscope using the characters suggested by Majerus & Kearns (1989) and weighted after anaesthetising them with ether for 30 seconds.

ii. *Reproduction and longevity of adults*: A pair of adults, within 24-hr of their emergence, were offered adequate quantity of alternative food or living aphids in a clean dry 5 cm diameter petridish. A corrugated piece of paper, as a means of oviposition site, was provided to each pair of adults. Observation was made at an interval of 12 hr for the laying of first batch of eggs. Thereafter observation was continued at an interval of 24 hr until the last batch of eggs was laid. These adults were continued to be kept in petridish and observed for the duration of their post-reproductive period until each of both the sexes died. This was repeated 10 times using each of the alternative foods and aphids.

iii. *Hatching success of eggs*: A batch of eggs, within 12 hr of laying, was removed to a 5 cm diameter petridish. Number of eggs hatched successfully, if any, was recorded during observations at an interval of 12- hr. Unhatched eggs were observed under the microscope for any sign of embryogenesis.

RESULTS

Development:

1. *Duration*: Duration of larval, pupal total development was longest by feeding DHBP and shortest by feeding AE+A. None of the larvae developed beyond second instar stage when offered SF. Mean duration of development of a larva, pupa and preadult stages (total) was significantly higher by feeding DHBP or AE in comparison to those which developed by feeding aphids but not so by feeding AE+A (Table 1).

Table 1. Mean duration of larval instas (L 1-4), pupa and total development of *M. sexmaculatus* obstained by feeding AE, DHBP, AE+A and their separate comparison with results obtained by feeding aphids (*A. craccivora*). Figures in a column followed by the same letter in parenthesis do not differ significantly at <0.05 level of probability.

Treatment (Food)	Duration of developement (days) (mean±se)			Mortality (%)
	L1-4	Pupa	Total	
Aphid	8.0 (a) ±0.67	4.2 (a) ±0.20	12.7 (a) ±0.77	44.50 (a)
AE	12.4 (b) ±0.52	5.9 (b) ±0.31	18.1 (b) ±0.69	57.82 (a)
AE+A	8.4 (a) ±0.53	4.1 (a) ±0.29	12.4 (a) ±0.49	46.14 (a)
DHBP	17.0 (c) ±0.47	7.2 (c) ±0.24	23.8 (c) ±0.44	75.0 (b)

ii) *Mortality*: Highest mortality in development was recorded in larvae which were offered DHBP (Table 1). Only 25% of these larvae reached adulthood. Adult emergence was 42.18% by eating on AE and 53.85% by eating AE+A.

iii. *Fresh weight of a developing larva and adult*: A first instar larva within 12-hr of hatching showed a mean fresh weight of 0.53 ± 0.002 mg. A full grown fourth instar larva, when stopped feeding, was significantly heavier by eating AE than the other alternative foods or aphids offered in this study (Table 2). Net gain in the fresh weight of larvae during its life and of adults on emergence which developed by feeding AE or DHBP were significantly different from each other and with those ate aphids. However, the larvae which ate AE+A did not show significant difference when compared with those fed on aphids alone (Table 2).

Table 2. Mean value of fresh weight of a fully grown larva (L4), and an adult at emergence (within 12 hr) and net gain in fresh weight of a larva in its life (NGL) of *M. sexmaculatus* when reared on aphid, AE, AE+A or DHBP. Figures in column followed by the same letter in parenthesis do not differ significantly at <0.05: Student 't' test.

Treatment (Food)	Fresh weight of larva and adult (mg)		
	L4	NGL	Adult
Aphid	9.57±0.45 (a)	9.52±0.45 (a)	8.04±0.43 (a)
DHBP	8.10±0.45 (b)	8.05±0.45 (b)	6.80±0.41 (b)
AE	11.90±0.60 (a)	9.70±0.51 (c)	9.70±0.51 (c)
AE+A	9.48±0.48 (a)	9.27±0.43 (a)	8.40±0.46 (a)

Reproduction

1. *Pre-oviposition delay*: Oviposition by an adult ladybeetle usually follows pre-oviposition delay of several days depending on the quality of habitat experienced by larvae and adults at emergence (Wellings, 1981). In the present study duration of pre-oviposition delay was shortest when adults were offered aphids but longer in adults which were offered alternative foods. DHBP or AE+A fed - adult females showed significant difference when compared to adults fed on aphids, but in case of AE-fed adults the difference was not significant (Table 3). Analysis of results among the adults fed on alternative foods did not show significant difference.

iii. *Oviposition period*: Adult females fed on aphids showed significantly longer oviposition period compared to those fed on alternative foods (Table 3). Among the alternative foods, DHBP - fed females showed significant longer oviposition period in comparison to females fed on AE pr AE+A but the difference was not significant when compared among AE and AE+A - fed females.

iv. *Number of eggs*: Adult females oviposited by feeding on alternative foods offered in this study but the number of eggs laid was significantly less compared to those females fed on aphids (Table 3). Among the females fed on alternative foods, DHBP - fed females laid significantly high number of eggs compared to AE - fed females but the difference was not significant in comparison to AE+A - fed females.

v. *Hatching success of eggs*: Hatching success of eggs from adults fed on aphids was significant higher compared to eggs of adults fed on AE or AE+A but the difference was not significant when compared to eggs of adults which fed on DHBP (Table 3).

Adult longevity:

AE or AE+A - fed adults showed significantly shorter longevity compared to those fed on aphids. However, no significant difference occurred between DHBP and aphid - fed adults, both males and females (Table 3).

Correlation of oviposition period and number of eggs laid

Mean oviposition period showed positive and significant correlation with the mean number of eggs laid when adults of *M. sexmaculatus* were offered aphid, DHBP, AE or AE+A foods ($r = 0.998$, $p<0.001$). Regression analysis showed linear relationship between the two factors ($y = 91.79+12.35x$).

DISCUSSION

Results suggest that a combination of AE+A was almost equal in nutritional value to aphids in terms of duration of development, number of adults emerging successfully after development, fresh weight attained by a fourth instar larva, net gain in fresh weight of larva in its life and of adult at emergence. The same did not hold good in respect of AE or DHBP. Soyabean food proved to be almost non - starter.

Results on reproduction and adult longevity suggest that adult beetles of *M. sexmaculatus*,

Table 3. Mean duration of pre-oviposition delay (POD), oviposition period, total number of eggs laid, hatching success of eggs and longevity of adult (AL) male and female of *M. sexmaculatus* when fed on DHBP, AE or AE+A and their separate comparison with those fed on aphids, *A. craccivora*. Figures in a column followed by the same letter in parenthesis do not differ significantly at <0.05 level: Student 't' test.

Treatment (Food)	POD (days)	Oviposition period (days)	No. of eggs	Hatching Success of eggs(%)	AL(days)	
					Female	Male
DHBP	14.75(a) ±1.60	43.75(a) ±7.22	255.0(a) ±71.07	47.71(a,c) ±3.61	75.0(a) ±6.65	66.75(a) ±7.35
AE	13.62(a,b) ±3.44	7.75(c) ±3.58	21.37(c) ±6.16	21.61(b) ±6.72	41.25(c) ±3.25	36.25(b) ±2.83
AE+A	18.4(a) ±0.87	10.72(c) ±2.11(c)	73.4(a) ±9.82	31.84(a,b) ±2.50	66.80(c) ±2.48	38.60(b) ±3.14
Aphid	9.56 ±2.54	67.5(b) ±24.6	815.60(b) ±211.60	52.0(c) ±5.07	98.0(b) ±12.76	80.0(a) ±11.34

when fed on DHBP, AE or AE+A showed significantly reduced level of performance in respects of pre-oviposition delay, oviposition period, number of eggs laid, their hatching success and longevity of adults compared to those fed on aphids. It is obvious, although, that natural food provides the best value for any cost effective performance of an organism, nonetheless, it is remarkable that DHBP, AE and AE+A foods induced some level of oviposition response.

Though heavier larvae and adults were produced by feeding AE but this food seems to be deficient in some vital ingredients, not yet known, which were possibly responsible for the weak ovipositional response in an adult female. On the other hand DHBP, though prolonged the duration of pre-adult development, caused greater oviposition response in an adult female Niijima *et al.*, (1986) found that the nutrition value of DHBP is almost equal to aphids and considered the former an effective substitutional diet for many aphidophagous Coccinellidae including *M. sexmaculatus*. But *Coccinella septempunctata bruckii* Mulsant, a native japanese ladybeetle, laid fewer eggs and had a longer duration of pre-oviposition delay by feeding DHBP. Remarkably the same subspecies could not be successfully reared in Finland on an artificial diet (Hamalainen & Markkula, 1972). This implies that adequate nutritional requirement alone possibly is not enough for a ladybeetle to cause oviposition. Balanced artificial diets used in many studies either failed to cause complete development (Smith, 1965; Hukusima & Takeda, 1975) and / or evoke adequate ovipositional response (Smirnoff, 1958; Kariluoto *et al.*, 1976). Some other factors like gustatory and mechanical properties may also influence the response of oviposition provided the nutritional supply is adequate. Inadequate ovipositional response of the Indian population of *M. sexmaculatus* compared to Japanese population when fed on DHBP could be another example of similar situation.

Results suggest that a combination of AE + A holds promise for use in short term culture of aphidophagous ladybeetles. DHBP is useful in captive culture of ladybeetle on a limited scale and for the maintenance of stock of adults in particular.

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Biodiversity in the Western Ghats - A Study with Reference to Moths (Lepidoptera: Heterocera) in the Silent Valley National Park, India)

George Mathew and V. K. Rahamathulla
Division of Entomology, Kerala Forest Research Institute
Peechi 680 653, Trichur, Kerala

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Abstract: Insect species diversity with reference to moths was studied in representative forest habitats (viz., disturbed but well regenerating and relatively undisturbed) in the Silent Valley National Park. Preliminary data suggested rich species diversity in well regenerating forests (3.42 & 1.9) as compared to those subject to disturbances like incidence of fire (0.43). Maximum number of moths collected belonged to the families Pyralidae, Noctuidae, Geometridae and Arctiidae. Some families like Lasiocampidae, Bombycidae and Gelechidae were only poorly represented. In general, the fauna bears a close resemblance to that of Sri Lanka although it is characterised by the presence of several endemic species having affinities with the Malayan elements. Altogether 318 species of moths belonging to 19 families were recorded in this study.

Key words: Lepidoptera, Heterocera, biodiversity, fauna, moths, Silent Valley National Park.

INTRODUCTION

The tropical rain forests resulting from about 60 million years of evolution, are by far the most stable and sensitive ecosystems as compared to the temperate forests which are of comparatively recent origin. Because of its complex nature, any disturbance in the habitat is likely to affect the delicate balance existing between its various components. Man-induced changes leading to modifications in the land, water, flora and fauna are among the major factors which upset this balance. As a result of disturbances in the biome, many species particularly the insects, become extinct. Since most of the tropical rainforests are located in underdeveloped or developing countries, lack of adequate scientific expertise is a major constraint in undertaking ecological studies in order to develop sound management strategies. As a result, the disappearance of many species remain undocumented even before establishing their economic importance. Therefore there is an urgent need to study the fauna in these regions (Wells *et al.*, 1983).

The Silent Valley National Park is a typical humid tropical rain forest in the Kerala part of Western Ghats and forms the core area of the Nilgiri Biosphere Reserve (Fig. 1). It is situated on a plateau about 1000 m above mean sea level and covers an area of 9000 ha. Because of climatic, edaphic and altitudinal gradients, the forests of Silent Valley exhibits considerable variations in the floristic composition, physiognomy etc. Four types of vegetations are encountered viz., (a) west-coast tropical evergreen forests (b) substropical broad-leaved hill forests, (c) montane wet temperate forests and (d) grasslands.

Our knowledge on the insect fauna of Indian forests is largely based on earlier studies by pioneer workers like Hampson, (1896-1899). Although a series of revisionary studies have been subsequently carried out from different geographical regions, no exhaustive survey has so far been carried out specifically from the various forests. This is particularly true with regard to the Western Ghat region which is noted for its richness in biodiversity.

Among insects, the moths belonging to Lepidoptera are economically very important as the primary herbivores in the forest ecosystem. They are diverse in their habits and are adapted

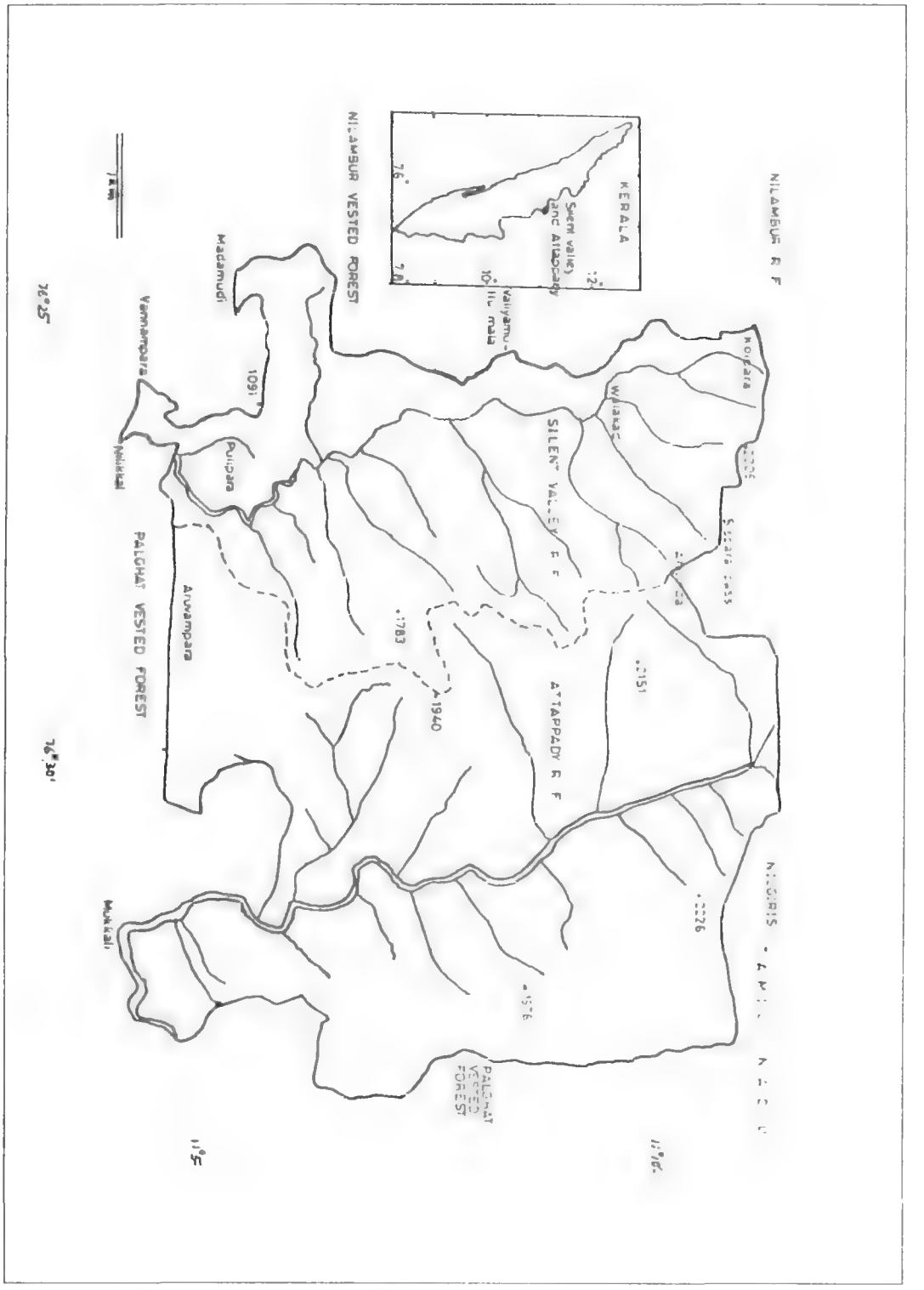

Fig. 1. Silent Valley and Surroundings.

to a variety of conditions. Being highly sensitive to changes in the environment, they are easily affected by even relatively minor perturbations in the habitat so much so they have been considered as indicators of environmental quality (Rosenberg *et al.*, 1986).

Materials and Methods

The moths were collected by using a modified Pennsylvanian-type light trap operated by a 6 V battery, with an automatic switching device to facilitate self operation of the trap in the deep forests at specified timings (Fig 2). An 8 watt UV tube was used in the trap for illumination. The trap was set up on a stand, about 1.2 m in height, on the ground in small forest clearings and the trap was operated overnight on all days (from 6 PM to 6 AM on the next day).

Insect diversity: In order to study the faunal diversity, regular sampling was carried out in four locations - 2 areas at Campsite and one each at Poochappara and Neelikkal within the Sanctuary. The insects collected on each night were sorted out into species and their numbers recorded. The first locality (Campsite, 1st area) represented a well regenerating forest which was subject to logging operations in the past. The second area at the Campsite was similar to the first but was subject to fire in the past . The third and fourth localities represented relatively undisturbed forest types. At each location monthly sampling was carried out for 5 successive days, serially from plot 1 to plot 4, for a period of 5 months. In addition to this, occasional sampling of fauna was carried out during visits to the other parts of the sanctuary.

The insects collected in this study were identified by reference to literature or by referring to the International Institute of Entomology, London.

Data analysis: For calculating the diversity index for the various localities, Shannon-weiner formula was used:

$$\text{Diversity index } (H^1) = -\sum_{i}^{s} P_i \ln (P_i)$$

where P_i is the proportion of the 'ith' species in the community, 'S' is the total number of species and ln is the log with base "e" (natural logarithm) Pielou, 1975).

Inorder to assess the overall similarity of different localities with respect to species diversity, the index of similarity (IS) was also worked out. A modified version of Jaccard's formula as suggested by Sorenson (1948) was used. According to this,

$$\text{the index of similarity (IS)} = \frac{2C \times 100}{(A + B)}$$

where

c = number of common species in two 'releves'.
A = total number of species in a Plot and
B = total number of species in another Plot.

Results

Altogether 318 species of moths belonging to 19 families could be collected and identified (Appendix). The families Geometridae, Noctuidae and Pyralidae contained maximum number of species recorded in this study.

Species diversity: The number of insects collected from the various Plots are given in Table 1. The highest number of insects collected was from Plot 1 and lowest from Plot 4 with the species diversity index ranging from 3.42 in Plot 1 to 0.43 in Plots 3 and 4 (Table 2). Although Plot 2 was adjacent to Plot 1, it registered a low value as compared to Plot I and this was attributed to the incidence of fire in the former in the previous years. However, with regard to Plots 3 and 4 where the structural quality of flora was far superior compared to the other Plots, the values obtained were quite low. The extact reasons for the low diversity in these Plots are not certain. Probably the influence of seasons (viz. full moon, new moon etc.) or the inappropriateness of sampling sites in these areas could have affected the trap catches. The influence of the above factors on trap catches could not be evaluated in this study since collections were made only for a short period of 5 months from January to May 1989.

Table 1. Number and percentage of species in each family collected from the study Plots (% given in brackets)

Families	Plot I	Plot II	Plot III	Plot IV	Total
Pyralidae	26 (21.14)	21 (25.30)	26 (32.13)	12 (30.00)	85
Geometridae	21 (17.07)	11 (13.25)	14 (17.28)	11 (27.50)	57
Drepanidae	-	1 (1.20)	-	-	-
Epiplemidae	-	2 (2.41)	1 (1.23)	1 (2.50)	4
Notodontidae	-	-	1 (1.23)	1 (2.50)	2
Lymantriidae	4 (3.25)	8 (9.64)	1 (1.23)	3 (7.50)	16
Arctiidae	22 (17.88)	15 (18.07)	11 (13.58)	9 (22.50)	57
Noctuidae	36 (29.27)	23 (27.73)	24 (29.63)	3 (7.50)	86
Sphingidae	9 (7.32)	1 (1.20)	1 (1.23)	-	11
Lasiocampidae	2 (1.63)	1 (1.20)	1 (1.23)	-	4
Saturnidae	3 (2.44)	-	-	-	3
Cossidae	-	-	1 (1.23)	-	1
Total number of species	123	83	81	40	

In order to judge whether the insects collected in the samplings fully represented the moth fauna, a collector's curve was prepared by plotting the number of insects collected upto the i-th period (i=1, 2, 3, 4, 5 month; pielou, 1974, p. 288). The curves (Fig.3) were found to rise continuously in all the localities indicating that the sampling was not sufficient and that further collections are necessary for getting a more complete estimate of the faunal diversity of these areas.

Table 2. Species diversity index for the four plots sampled

Plot No. and locality	No. of families	No. of species	No. of individuals	Species diversity index
Plot 1	8	123	580	3.42
Plot 2	9	83	319	1.9
Plot 3	10	81	327	0.43
Plot 4	7	40	180	0.4

Family diversity: The relative abundance of the various groups of moths was another aspect studied. Maximum number of families was recorded in Plot 3 (10; Poochappara) followed by Plot 2 (9; Campsite). Least number recorded was for plot 4 (7; Neelikkal). In all the four Plots, the families Pyralidae, Noctuidae, Geometridae and Arctiidae were the dominant groups (Fig. 4). Certain families like Sphingidae, Lasiocampidae, Saturnidae and Cossidae were very scarce in the trap catches. The representation of certain families in the various Plots was interesting. The family Saturniidae was represented only in Plot 1 and Cossidae in Plot 3. The distribution of some of these families might prove to be of importance in that their occurrence could probably be associated with specialities in the floral composition in a particular habitat.

Table 3. Similarity index values for the four localities studied

	Similarity indes values			
Plot Nos.	I	II	III	IV
I		61	64	42
II			60	59
III				56

Faunal affinities and endemism in the fauna: The faunal similarity of the four Plots was studied and the indices of similarity are given in Table 3. The Plots 1, 2 and 3 and more of less same value, compared to that obtained for Plot 4 which was low. The resason for this is not known but could be due to differences in the floral composition. Further studies are needed to ascertain this aspect.

DISCUSSION

The study has indicated that the Heterocera of Silent Valley is rich and diversified. Altho-

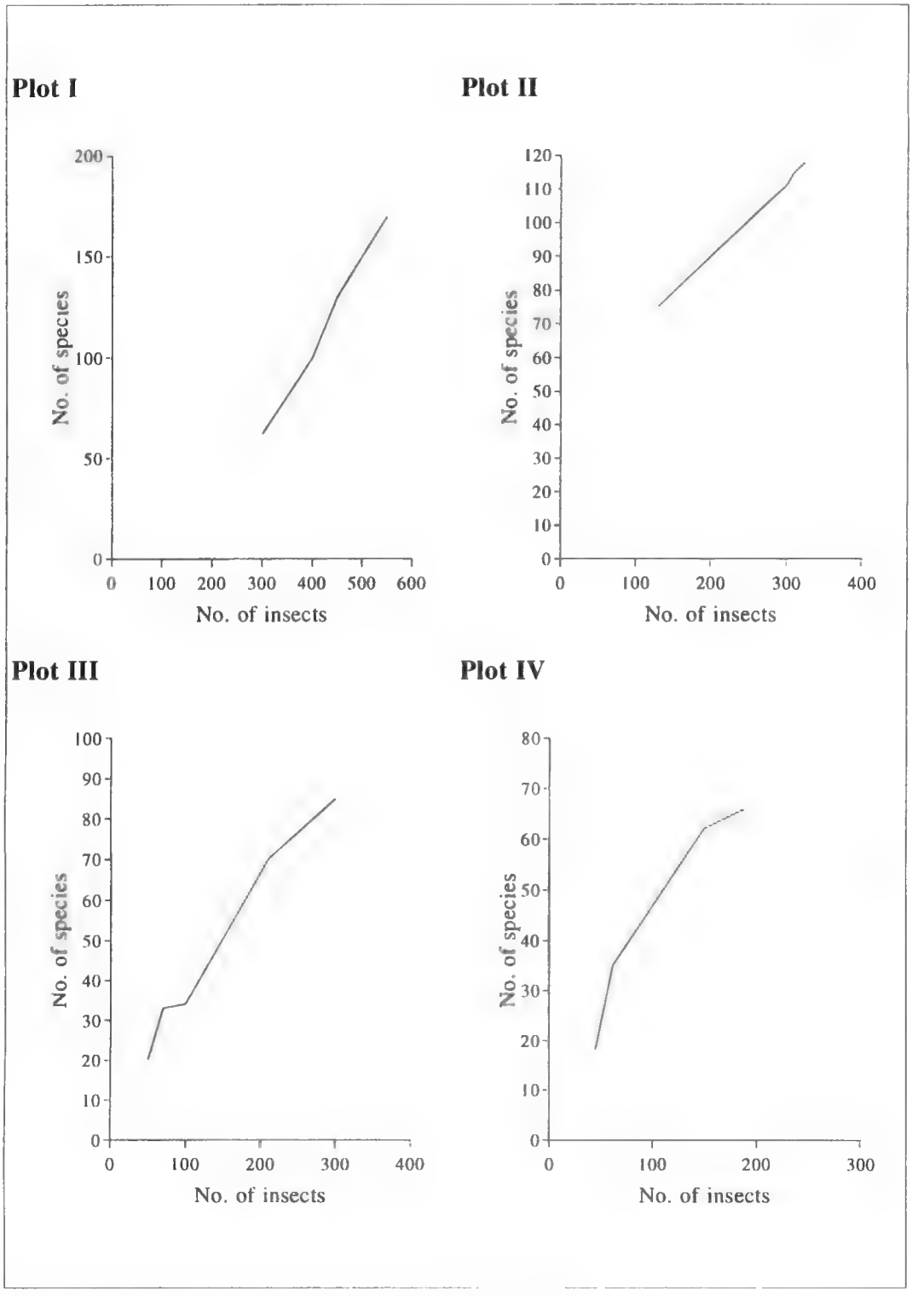

Fig. 3. Collector's curve for Plots I, II, III and IV.

ugh collections could be made only for a short period of 5-6 months in a year (due to inaccessability to this area during the rainy seasons as a result of landslides, tree fall, etc.) altogether 318 species of moths have been recorded. The findings of this study indicate that, well regenerating forests are rich in species diversity (3.42). The adverse effect of disturbance on fauna was also shown by the drastic reduction in the diversity index in fire affected forest patch (1.9). However, the fact that further samplings are necessary, is indicated by the collector's curve, which shows an upward rise. The species diversity indices obtained for the various localities therefore do not actually indicate the exact status of diversity and the values obtained here are of an indicative nature only.

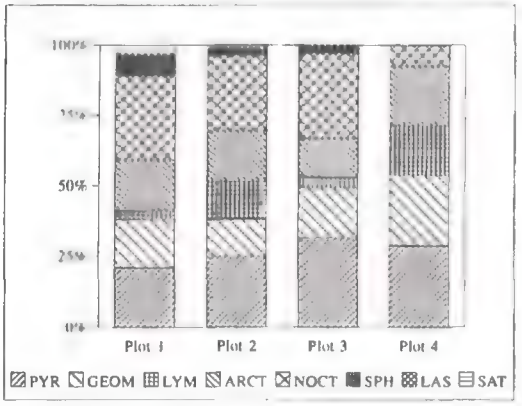

Fig. 4. Propagation of various major taxonomic groups in four sampling sites. (PYR-Pyalidae; GEOM-Geometridae; SPH-Sphingidae; LYM-Lymantriidae; ARCT-Arctiidae; NOCT-Noctuidae; SAT-Saturnidae).

The fauna of Silent Valley bears a close resemblance to that of Sri Lanka, although the latter is characterised by the occurrence of several endemic genera and disjunct species groups which do not have any relatives in S. India (Larsen, 1987). The low land evergreen forests of Silent Valley have a good representation of South Indian species although the forest patches at higher altitudes as well as the sholas contain several species which bear a close resemblance to that of Sundaland although they have developed into distinct races over years of isolation. Holloway (1974) and Larsen (1988) are of the opinion that Indian fauna is one largely formed as a result of displacement by invaders from other regions of the Oriental region, after its separation from Gondwanaland and merger with Asia. Most of the endemic species in the Western Ghats had their origin elsewhere in the Oriental region and are still surviving in isolated habitats. The moths *Loepa sikkima, Trabala ganesha, Oxyambulyx subocellata, Theretra nessus, Macroglossum aquila, Tarsolepis rufobrunnea malayana, Phalera sundana, Cyana perornata, Eliema tetragona, Oeonistis entella, Spilosoma anada, Tridrepana fulvata,* etc., (Barlow, 1982) are some of the species having Malayan affinities recorded from the Nilgiri Biosphere Reserve. A small fraction of insects are having Palaearctic (*Borbacha* sp., *Eumelia rosalia, Ozarba punctifera, Rhodogastria* sp., *Euproctis bipunctapex*), Australian (*Maceda mansueta, Pyrausta phoenicealis, Crocidolomia* sp.), and Ethiopian (*Pingasa ruginaria, Britta* sp., *Sauris* sp.) affinities.

Fig. 2. Battery operated light trap used for moth sampling.

Man induced disturbances are among the main factors that affect the sustenance of many natural communities. Although extinction of species is supposed to create diversity due to

diversification and adaptation of the surviving ones, destruction of species is unlikely to generate very much diversity in the rainforests because of its complex structure (Turner, 1984). The occurrence of a rich and diversified fauna in some parts of Nilgiri Biosphere region was largely attributed to the conservation of forests in this region (Larsen, 1987, 1988). Conservation of the natural habitats is very essential for the existence of many species of lepidopterans. The survival of a large number of endemic species in the Silent Valley area warrants frequent monitoring of the ecological processes besides adoption of appropriate conservation strategies in order to safeguard its rich genetic diversity.

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Appendix - List of moths collected from Silent Valley National Park

HETEROCERA (MOTHS)
Drepanidae
Teldenia Sp
Phalacra vidhisara Walker
Tridrepana fulvata Snellen
Thyrididae
Banisia myrtaea (Drury)
Pyraloidea
Tyndis hypatialis (Swinhoe)
Prophtasia pyrostrota (Hampson)
Crocidolomia pavonana (Fabricius)
Culladia admigratella Ragonot
Ancylolomia chrysographella Kollar
Musotima suffusalis Hampson
Cataclysta blandialis Walker
Pycnarmon caberalis Guenee
Agrotera basinotata Hampson
Actholix flavibasalis Guenee
Pagyda salvalis Zeller
P. traducalis Zeller
Cnaphalocrocis sp.
Marasmia venilialis Walker
Syngamia abruptalis Walker
S. abjungalis Walker
Aethaloessa floridalis Zeller
Dichocrocis punctiferalis Walker
D. plutusalis Walker
Hyalabathra miniosalis Guenee
Paradomima distorta Moore
Botyodes asialis Guenee
Sylepta lunalis Guenee
S. quadrimaculalis Kollar
S. derogata Fabricius
S. tibialis (Moore)
S. balteata Fabricius
Agathodes ostentalis Hubner

Artroschista hilaralis Walker
Parotis marinata Fabricius
P. marginata Hampson
P. vertumnalis Guenee
Glyphodes stolalis Guenee
G. itysalis Walker
G. bivitralis Guenee
G caesalis Walker
G. bicolor Swinson
G. indica Saunders
Phlyctaenia tyres Cramer
Eucalsta filigeralis Leaderer
E. defamatalis Walker
Nausinoe perspectata Fabricius
N. geometralis Guenee
Leucinodes orbonalis Guenee
Crocidophora ptyophora Hampson
Pachyzanea licarsisalis Walker
P. cynaralis Walker
Eutectona machaeralis Walker
Maruca testulalis Geyer
Filodes fulvidorsalis (Hubner)
Daulia afralis Walker
Pyrausta phoenicealis Hubner
Ephestia cautella (Walker)
Nephopteryx artisquamella Hampson
Epicrocis lateritialis Walker
Hyalospila leuconeurella Ragonot
Etiella zinckenella Treitschke
Europheza? subarcella Meyrick
Geometridae
Astygisa Sp.
Fascellina plagiata Walker
F. chromataria Walker
Gasterocome pannosaria Moore
Corymica pryeri Butler
Hyperphyra cyanosticta Hampson
Luxiaria hypaphanes Hampson
Lusiaria postvittata Walker
L. sp? subrasta Walker
Buzura sp. ? suppressaria Guenee
Cleora sp. alienaria Varren
Cleora sp. falculata Fletcher
Cleora sp.
Menophra ? inouei Sato
Eurytaphria sp.
Catoria sp.
Ectropis sp. ? *breta Swinhoe*
Ectropis bhurmitra Walker
Ectropis indiststincta Hampson
E. ? boarmiaria Guenee
E. inconclusa Walker
Medasina sp.
Hypomecis pallida Hampson
H. sp. nr. dentigerata Warran
?Hypomecis sp.
Scopula sp. ? nr. *pulverosa Prout*
Scopula sp.
Trimandra sp. ? *nelsoni* Prout
Ptochophyle togata Fabricius
Antitrygodea divisa Walker
Antitrygodes culeinea Walker
Petelia sp. of *medardaria Herrich-Schaffer* group-
Hypochrosis festivaria Fabricius
H. pachiaria Walker
H. sp. ? *abstractaria* Walker
Sabaria costimaculata Moore
S. sp. nr. *rondelaria* Fabricius
S. incitata Wit.
Semiothisa sp. prob. *nora* Walker
Semiothisa triangulata Hampson
S. ferruginata Moore
S. myandaria Walker
S. quandraria Moore
Semiothisa sp.
Ourapteryx marginata Hampson
Thinopteryx crocoptera Butler
Heterostegane subtessellate Walker
Heterostegane sp.
Borbacha sp. ? paradarica Guenee
Lomographa sp. ? *simpliciaria* Walker
Abraxas sp. of *poliaria* Swinhoe group
A. sylvata Scop.
A. sp. near *latizonata* Hampson
Scardamia rectilinea Warren
Plutodes sp? *discigera* Butler
Plutodes sp
Ecliptopera subapicalis Hampson
E. dissecta Moore
Oenospila quadraria Guenee
Vliocnemis partita Walker
V. biplagiata Moore
Archaeobalbis cristata Warren
Pachyodes luteipes Felder
Hemithea sp. *graminea* Hampson
Comibaena inductaria Guenee
C. integranota Hampson
Neromia carnifrons Butler
Pingasa ruginaria Guenee
P. chlora Cramer
Eumelia rosalia Stoll
Sauris sp.
Elpho sp.
Polynesia sunandava Walker
Xanthorhoe molata Fleder
Callidulidae
Cleoseris catamitus Hubner
Uranidae
Psuedomicronia sp.
Epiplenidae
Epiplema? moza Butler
Dirades unicauda Dudgeon
D. leucocera Hampson
D. theelata Guenee
Epiplema albida Hampson
Noctuidae
Neochera dominia Cramer
Condica illecta Walker
Achaea janata Fabricius
Ercheia cyllaria Cramer
Callopistria rivularis Walker
Pericyma umbrina Guenee
Hypena? subalbida Bethune-Baker
Paracrama latimargo Warren
Earias flavida Felder
Maceda mansueta Walker
Labanda fasciata Walker
Nycteola grisea Hampson
Blenina lucretia Dalman
B. sp. nr *lichenosa* Moore
Lophoptera illucida Walker
Hadennia sp. ? *prunosa* Moore
Rhynchina curvilinea Hampson
Britha pactalis Walker
Pseudogyrtona perversa Walker
Eustrotia marginata Walker
Ozarba sp. ? *punctigera* Walker
Maliattha erecta Moore
Corgatha semiparata Walker
Anomis sabulifera Guenee
A. figlina Butler
Tinolius quadrimaculatus Walker
Nola cingalesa Moore
Masalia bimaculata Moore
Janseodes melanospila Guenee
Sasunaga sp. ? *tenebrosa* Moore
Mythimna curvilinea Hampson
M. reversa Moore
M. vittata Hampson
Mythimna sp.
Tiracola plagiata Walker
Athetis renalis Moore
Condica illecta Walker
Odontodes aleuca Guenee
Pseudathyrma hubo Hubner
Xenotrachea albidisca Moore
Mudaria (= Plagideicta) sp. ?*leprocticta* Hampson
Digama marchalli Guerin
Carea endophae Hampson
Ericeia sp. ? inangulata Guenee
Rhesala moestalis Walker
Saroba pustulifera Walker
Hydrillodes sp. prob. *nilgirialis* Hampson
Thyas honesta Hubner
Rhytia hypermnestra (Stoll.)
Elygoa materna (Linnaeus)
Othreis fullonia (Clerck)
Episparis liturata (Fabricius)
Targalla ludatrix (Walker)
Erebus caprimulgus
E. hieroglyphica Drury
E. ephesperis
Arte sp.
Spodoptera mauritia Boisdual
Maurilia sp.
Hypospila bolinoides Guenee
Bocana manisestalis Walker
Batracharta variegata Walker
Arthisma scissralis Moore
Cerynea ustula Hampson
Cocyparis insolituta Walker
Tagiades litigiosus Moschler
Sarobides sp.
Oxyode sp.
Arctiidae
Amata extensa Walker
Argina syringa Cramer
A. astrea Drury
Pericallia sp. of *ricini* Fabricius complex
Eilema tumida Walker
E. sp. ? *obliterana* Walker
Eilema tetragona Walker
? Eilema sp.
Eilema sp.
Macotasa sp. ? *nubecula* Moore
Nilgiricola sicciana Hampson
Siccia taprobanis Walker
Cyme gratiosa Guerin - Meneville
Asura metamelas Hampson
A. sp. ? *obsoleta* Moore
A. arcuata Moore
A. rubricosa Moore
Asura sp.
Eugoa sp. of *hipunctata* Walker complex
Spilosoma sp. ? *mona* Swinhoe
S. ananda Roepke
Spilosoma sp.
Pangora matherana Moore sp.
Paraplastis hampsoni Swinhoe
Lemyra sp.
Paraona splendens Butler
Ceryx transitiva Walker
Evessa sp.
Oeonistis entella Cramer
Cyana bianca Walker
C. malayensis Hampson
Cyana sp.
Rhodogastria astveus Drury
Asota plana Walker
Asota producta Butler
Nyctemera coleta Cramer
N. adversala Shallev
N. baulus Boisduval
Euchromia polymena Linn.
Syntomis thoracican Moore.
Lymantriidae
Cispia charma Swinhoe
Lymantria sp. probably *Kanara* Collenette
L. todara Moore
Aroa sp. of *plana* Walker complex
Redoa sp.
Euproctis hipunctapex Hampson
E. fraterna Moore.
E. percnogastor Collenete
E. semisignata Walker
Laelia? colon Hampson
Cispia charma Swinhoe

Notodontidae
Poliostauropus grisea Hampson
Tarsolepis rufobrunnea Nakamura
Phalera javana Walker
Dadusa nobilis Walker
Cerura sp.
Sphetta apicalis Walker
Limacodinae
Miresa argentifera Walker
Caissa gambita Hering
Scopelodes sp prob. *venosa* Walker
Susica sp. ? *himalayana* Holloway
Poliostauropus grisea Hampson
Bombycidae
Penicillifera sp. prob. *apicalis* Walker
Tortricidae
Nenomoshia poetica Meyrick
Olethreutes paragramma Meyrick
Lasiognatha mormopa Meyrick
Dactyloglypha harmonica (Meyrick)
Udea ferrugalis Hubner
Bostra indicator Walker
Thylacoptila paurosema Meyrick
Adoxophyes revoluta Meyrick
Archips sp.
Gelechidae
Dichomeeris sp.
Cossidae
Xyleutes sp.
Zeuzera indica Herrich Schaffer
Phragmataecius impura Hampson
Saturnidae
Argema maenas Doubleday
Attacus atlas Linnaeus
Loepa sikkima Moore
Lasiocampidae
Cyclophragma sp.
Sphingidae
Acherontia lachesis Fabricius
A. styx. Westw.
Meganoton sp.
Oxyambulyx sp
Agrius sp.
Hippotion boerhaviae Fabricius
Theretra sp. *costanea* Moore
Macroglossum aquila Biosduval
Rhagastis sp. ? castorWalker
Acosmeryx shervillii Boisduval
Herse convoluvi Linn.

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Circadian Rhythms of Sugar Levels in Fifth Instar Semilooper Caterpillars of *Achaea janata* Linn.

Annie John
Sree Chitra Institute of Medical Sciences & Technology
Statelmond Palace, Poojappura, Trivandrum

D. Muraleedharan
Department of Zoology, University of Kerala
Kariavattom - 695 581, Trivandrum, India

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Abstract: Rhythmic levels of trehalose and glycogen in haemolymph and FB of the Semilooper Caterpillar, *Achaea janata* revealed its endogenous circadian pattern in conformity with a similar feeding rhythm. The rhythms of trehalose and glycogen entrained in LD and free ran in LL and DD with phase shifts. They exhibited mainly bimodal peaks - one in the photophase and the other in the scotophase. The range of larval haemolymph trehalose was between 352-1339 mg/100ml, fat body trehalose 266-725 µg/100 mg, and fat body glycogen 591-5406 µg/100 mg. The haemolymph trehalose peaks in the scotophase were similar to the peaks of the feeding rhythm (at hour 04 and at hour 20 on all days). Glycogen and trehalose seem to be mutually related regarding their interconversions in FB and haemolymph, maintaining homeostasis.

Key words: *Achaea janata*, Larva, Prepupa, Trehalose, Glycogen, Rhythm, Haemolymph, Fat body.

INTRODUCTION

A universal and ubiquitous phenomenon in most organisms is the existence of metabolic rhythms. The imporance of sugars as a reserve food material for various animal organisms is well known. Fat body (FB) is the principal centre of carbohydrate metabolism (Chefurka, 1965; Wyatt, 1967) and is analogous to the hepatic functions in vertebrates (Candy and Kilby, 1961). The non-reducing disaccharide trehalose (1-[α-D-Glucopyranosyl] α-D Glucopyranoside) is the principal sugar in the haemolymph of many different insects (Wyatt, 1961). Thus it is of interest to compute what fraction of an insect's carbohydrate reserve is in the form of trehalose and glycogen in the haemolymph and FB and how this fluctuates rhythmically during development and under varying photoperiods (12L:12D:, LL-24, hrs, DD-24 hrs) to reveal its endogenity. Feeding being the beginning of a chain of physiological and metabolic events, the feeding pattern was also elucidated under LD 12:12, to correlate the intake of food with the carbohydrates in FB and haemolymph.

MATERIALS AND METHODS

Newly ecdysed fifth instar larvae of *A. janata* L. (Lepidoptera : Noctuidae) were collected from the laboratory colony of fourth instar larvae which molted within a period of ± 2 hours, for the biochemical investigations. The caterpillars were then reared in three different transparent containers, fed *ad libitum* on Castor leaves and maintained under different photoperiods (12L : 12D); complete light (LL) and complete darkness (DD) in photoperiodic cham-

bers in the Laboratory (29°c ± 3°c, r. h. 90 ± 3%) (Annie John and Muraleedharan, 1989). Haemolymph and FB were randomly collected from thecaterpillars at ecdysis and thereafter at every 4 hr time interval throughout the larval and prepupal period and processed for trehalose and glycogen estimations.

Collection of Haemolymph and FB

Haemolyph (10 µl) was obtained by a prick near the base of the leg of the caterpillar and drawn up into a micropipette. Thereafter the larva was dissected out for the FB which spreads out as wavy sheets of white or yellow ribbons along the whole length of the body cavity. Fat body removed, was dried in vials at 60°c in the oven after which the desired weight (2 mg) was taken. FB was scarce on day I, so no estimation could be done on that day.

Trehalose Estimation in Haemolymph and FB

Haemolymph and FB were then processed according to the method of (Wimer *et al.*, 1970) with minor changes, The dried samples were then subjected to acid-alkali hydrolysis and tested for trehalose using 0.2% anthrone reagent (Seifter *et al.*, 1967), and quantitated using a spectronic-20 (Bausch and Lomb) at a wavelength of 6.30 nm for trehalose.

Glycogen Estimation in FB

Dried FB was digested with 30% Koh and estimated using 0.2% anthrone reagent (Seifter *et al.*, 1967) and quantitated using a spectronic-20 (bausch and Lomb) at a wavelength of 620 nm.

Statistical Analysis

A mean of five separate values denoted the value of each time of the day. Statistical significance was calculated using student's-t test and Anova - CRD for the correlation analysis (Snedecor and Cochran, 1967).

Feeding Rhythm: Feeding rhythm of the fifth instar larva was also elucidated adopting the method followed by George and Muraleedharan (1990) by tracing the outline of castor leaves on graph papers. Haemolymph trehalose levels with feeding levels were also analysed using student's-t test (Snedecor and Cochran, 1967).

RESULTS

The diverse patterns of trehalose and glycogen in haemolymph and FB portrayed their circadian rhythmicities as well as correlated developmental events with metabolic and physiological changes in insects. They exhibited mainly bimodal peaks - one in the photophase and the other in the scotophase. The rhythms of trehalose and glycogen in *A. janata* entrained under LD and free ran in LL and DD with phaseshifts (Fig. 1-3). Significant levels of trehalose and glycogen are marked with an asterisk in the graphs.

Entertainment of the rhythm

Haemolumph Trehalose: Larval haemolymph trehalose peak on Day 1 was at hour 20 in LD, persisted in DD but in LL a forward phaseshift of 4 hours occurred. However, an additional peak at hour 04 was perceived in DD. In Day 2, larvae under LL and DD, the peaks persisted as in normal but an additional peak was seen at hour 20 in DD. In day 3, under LL, a bimodal circadian rhythm was observed with their peaks at hour 20 and at hour 04 as in normal larvae but in DD the peaks backshifted 8 hours from peak to hour 12 and 4 hours from peak to hour 24 with respect to the normal cycle. In Day 4, the peaks persisted at hour 16, in LD, LL and DD, but in DD an additional peak was seen at hour 04. In Day 5, the peak at hour 08 in LD was not present in LD and DD and so this can be assumed as an insignificant peak and the rhythm seemed to damp out at the end of the prepupal period, may be the animal becomes aperiodic in LL and DD at the end of the cycle (Fig. 1).

Fat body Trehalose: With regard to larval FB trehalose, in LL, the peak in Day 2 phase shifted 8 hours forward with respect to the normal cycle but it persisted in DD at hour 20. In day 3, the bimodal peaks at hour 12 and hour 24 in LD, persisted in LL but in DD it persisted at hour 12 but the second peak extrap-

olated backward 4 hours ie. to hour 20. In Day 4, the single peak persisted at hour 08 in LL but in DD, the first peak phaseshifted 4 hours forward while the second peak persisted at hour 20 as in the normal. In Day 5, all peaks were at the same time ie. at hour 12 in LD, LL and DD (Fig. 2).

Fat body Glycogen: In LL and DD, with regard to fat body glycogen in peak in Day 2 persisted at hour 20; in day 3 under LL the peak backshifted 4 hours to hour 12 and the second peak 8 hours backward to hour 20 with respect to the normal but in DD it shifted 8 hours backwards to hour 08 and 4 hours backward to hour 24 with respect to LD. In day 4, under LL, the peaks shifted 4 hours backwards to hour 08 and 8 hours backwards hour 20 with respect to LD 12:12 and in DD only a single peak was seen, at hour 20 similar to as in LL which backshifted 8 hours with respect to the normal (Fig.3).

Correlation analysis for the comparison of effects of various photoperiodic treatments on trehalose and glycogen titers were also tabulated with their significances (Table 1).

Table 1.

Treatments	Haemolymph Trehalose	Fat body Trehalose	Fat body Glycogen
LD/LL	0.5702*	0.0715	0.2385
LD/DD	0.4955*	0.1442	0.1271
LL/DD	0.2820*	0.0375	0.2010

* $p < 0.01$

The range of larval haemolymph trehalose level was between 352-1339 mg/100 ml, fat body trehalose 266-725 µg/100mg and fat body glycogen 591-5406 µg/100 mg under LD 12:12. Glycogen and trehalose seem to be mutually related regarding their interconversions in FB and haemolymph maintaining an equillibrium between these reserves. It was noticed that trehalose titer were low in FB compared to that in the haemolymph and glycogen level was always higher than trehalose in FB but lower than trehalose level in haemolymph (Fig. 4).

Feeding Rhythm: The haemolymph trehalose peaks in the scotophase correlated with the peaks of the feeding rhythm at hour 04 and at hour 20 on all days, after which feeding gradually ceased and the larvae entered in to coccoon spinning and preparation. Maximum food consumed was 2463 mm^2 on Day 3, revealing its voracious feeding nature (Fig.5). Significance in the feeding levels in comparison with haemolymph trehalose levels are marked with an asterisk in the graph.

DISCUSSION

Circadian rhythm, an endogenous biological rhythm with a period approximately the length of a day is ubiquitous among the physiological organization of eukaryotes forming the basis of daily rhythm in animal life (Chiba and Tomioka, 1987). The diverse patterns of trehalose and glycogen portrayed their circadian rhythmicities. Regarding larval haemolymph trehalose in LD, the peaks appear in late photophase and in the early and late scotophase where by the latter coincides with the feeding rhythm showing its correlation, which might in turn be affected by diet changes in the secretion of hormones by the neurosecretory cells. In LL and DD most of the peaks occur during the late photophase and late scotophase. Such peak activities of carbohydrates may correlate locomotor activity to feeding activity; being the caterpillars are nocturnal. With regard to larval FB trehalose in LD, diphasic pattern activity is exhibited leaking at early scotophase and early photophase while in LL it appeared in early photophase and late scotophase and in DD at noon and early scotophase. With regard to larval FB glycogen, in LD, bimodal peaks appear in late photophase and late scotophase but in LL and DD they appear in early photophase and early scotophase. The significant correlation of glycogen and trehalose levels shows that change of photoperiod affected these levels, since physiological functions may be impaired or changed when the circadian system is driven away from resonance with its entertaining light/dark cycle (Masaki, 1984; Nunes and Veerman, 1984) and this exogenous modification was classically termed

"masking effect" (Aschoff, 1960).

No damping and abolishing of the rhythm in LL was seen in *A. janata* as observed in other insect rhythms (Mizoguchi and Ishizaki, 1984; Weidenhann and Loher, 1984;Saunders, 1987). Peaks of activity did not occur at the same time in all the days in *A. janata* but resulted in phaseshifts even in the same photoperiod as observed in *Drosophila* larvae too (Rensing, 1969). Changes in hormone level (Rensing, 1971) or different light intensities (Tilgner, 1967) or changes in the course of ontogeny (Gray and Bacharach, 1967) are correlated with changes in frequency and phase of the circadian rhythm (Rensing, 1971). Thus the phaseshifts might be attributed to the different physiological and developmental stage the insect experiences during this prolonged period of growth affected by different photoperiods. In constant conditions the animal responds with phaseshifts in an effort to synchronise its rhythm with the apparently changed L/D cycles. When these rhythms persisted in constant conditions with phase adjustments, it indicated that organisms possess some means of timing these periods and have a clock like mechanism, i. e. "living clocks". Bimodal peaks observed in the trehalose and glycogen rhythms in haemolymph and fat body of *A. janata* were also observed in the cells of the endocrine glands and fat body cells of larval *Drosophila*, in oxygen consumption rhythms of larval *Drosophila melanogaster* (Hardeland and Stange, 1971) and also in other species (Weidemann and Loher, 1984; Clopton, 1984; Gouteux and Monten, 1986). Bimodal peaks are truly endogenous and free run in constant conditions (Chiba, 1964; Nayan and Sauerman, 1971). Bimodal peaks in the haemolymph trehalose coincided with bimodal peaks of the feeding rhythm of the fifth instar larva in the scotophase being the caterpillars nocturnal. Other coincidences of rhythms reported are oxygen consumption and locomotor rhythms in *P. americana* (Janda and Merciak, 1957) and in *Anopheles gambiae* (Jones *et al.*, 1967) locomotor activity and feeding in *P. americana* (Liption and Sutherland 1970) and oviposition and locomotor activity in *Anopheles gambiae* (Jones *et al.*, 1967). The rhythmicity displayed by the corpus allatum and brain neurosecretory cells in *A. janata* (George and Muraleedharan, 1990; Mohankumar and Muraleedharan, 1985) may be perhaps influencing the diel changes in the feeding and carbohydrate rhythms, since the titre of various hormones also varies rhythmically across the day. Secretary circadian rhythms of carbohydrates in *A. janata* (Raman and Muraleedharan, 1989) exhibited low activity at 12 noon and high activity during scotophase and the latter may be correlated to feeding and trehalose rhythms in *A. janata*. Coincidences of the trehalose and feeding peaks reveal that the precursors of trehalose synthesis are obtained from the diet in *A. janata*. The switch may occur as a consequence of the hormonal events that follow feeding (Langley, 1966; Gee, 1975).

Fat body being a depot for glycogen storage (Chefurka, 1965; Wyatt, 1967; Candy, 1961; Murphy AND Wyatt, 1965) is undoubtedly a precursor for trehalose synthesis besides its function as a metabolic energy reserve. A hike in blood trehalose is accompanied by a concomitant fall in glycogen level in FB to maintain homestasis. The reduction of trehalose level during prepupation towards L-P ecdysis may be due to its requirement of glucosamine for chitin synthesis (Zaluska, 1959; Candy and Kilby, 1962) besides normal metabolic requirement as well as due to cessation of feeding or trehalose converted to other sugars (Woodring *et al.*, 1977) or its utilisation for the energy consuming processes during spinning and cocoon formation. Elevated haemolymph trehalose levels in larva may be correlated with decreased capacity of FB to retain intracellular trehalose (Jungries and Wyatt, 1972). Circulating levels of trehalose increases rapidly in response to experimental handling of the insect (Mathews and Downer, 1973; Hanaoka and Takahashi, 1976) with additional trehalose resulting from rapid activation of glycogenolysis in FB (Mathews and Downer, 1974) which

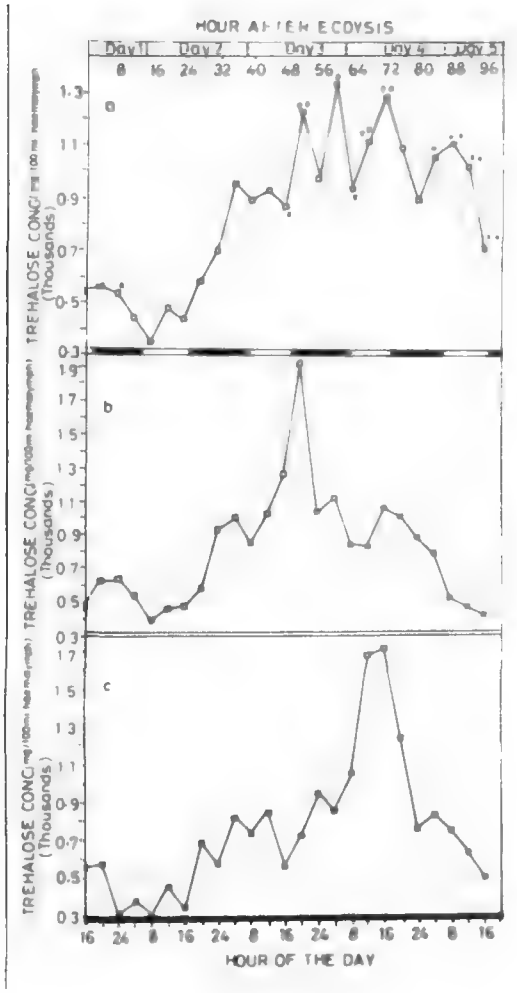

Fig 1

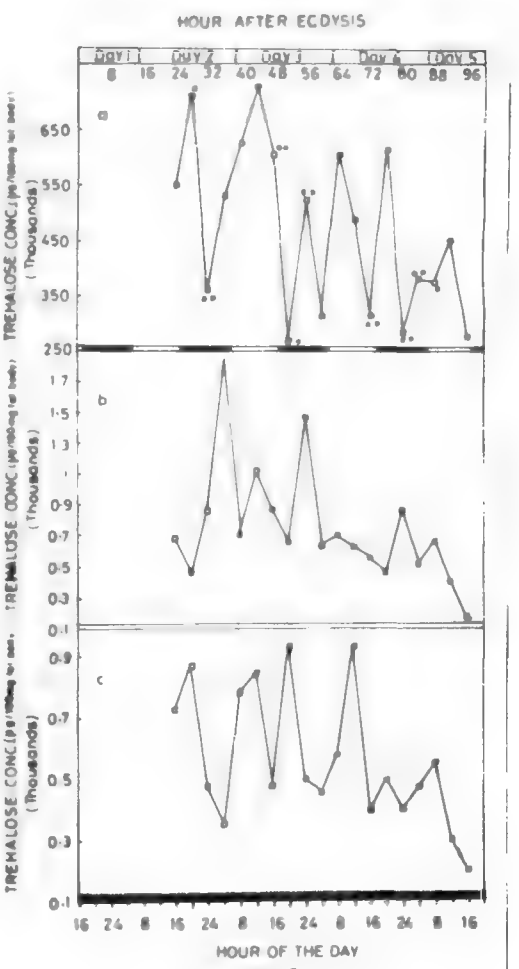

Fig. 2

Figure 1. Haemolymph Trehalose levels in fifth instar larva and prepupa of *A. janata* in different photoperiods (1) LD (b) LL (c) DD ($p^{**} < 0.01$ and $p^{*} < 0.05$).

Figure 2. Fat body Trehalose levels in fifth instar larva and prepupa of *A. janata* in different photoperiods (a) LD (b) LL (c) DD ($p^{**} < 0.01$ and $p^{*} < 0.05$).

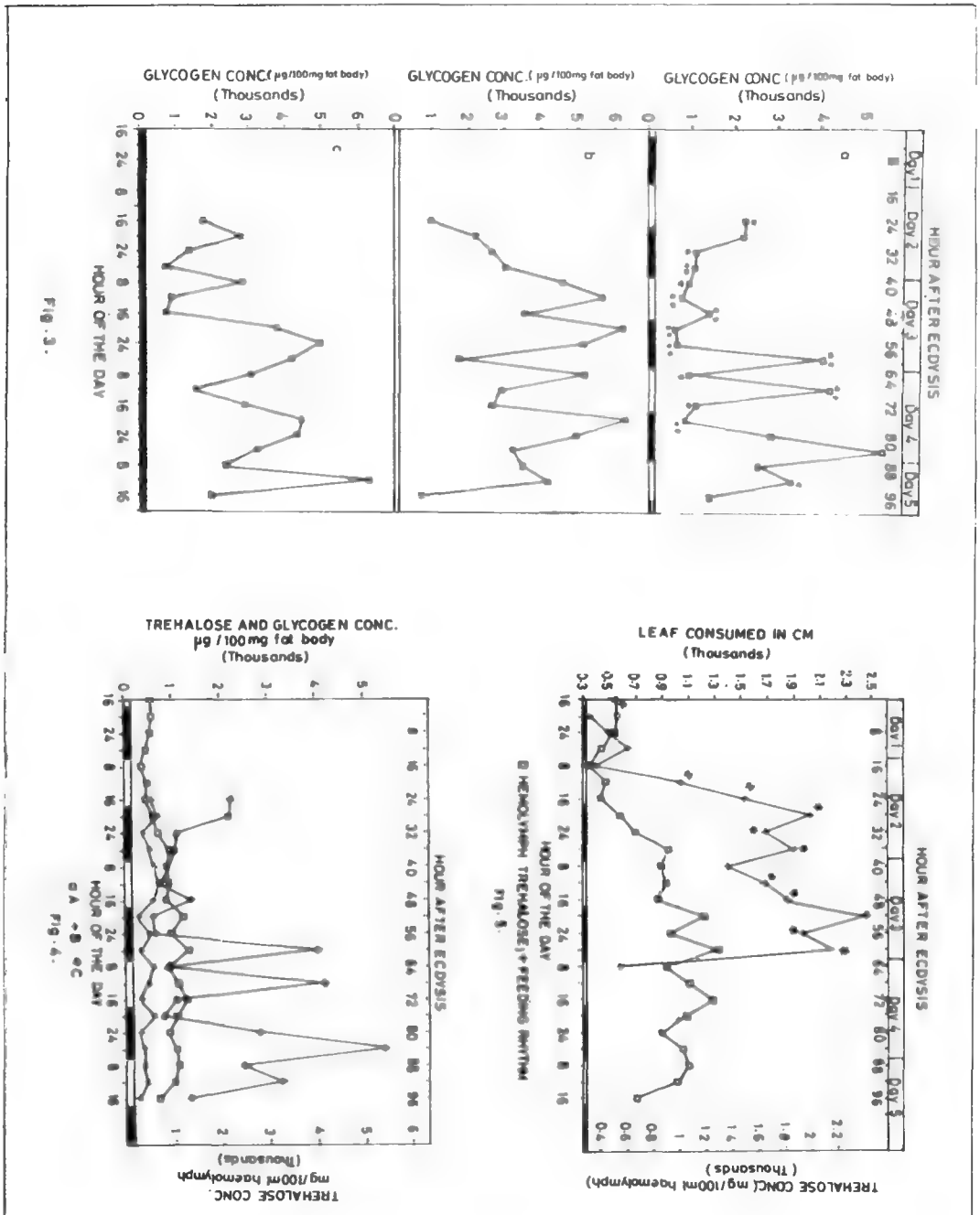

Figure 3. Fat body glycogen levels in fifth instar larva and prepupa of *A. janata* in different photoperiods (a) LD (b) LL (c) DD ($p^{**} < 0.01$ and $p^{*} < 0.05$).

Figure 4. Relationship of haemolymph Trehalose levels (A), fat body trehalose levels (B) and glycogen levels (C) in fifth instar larva and prepupa of *A. janata* in LD 12:12.

Figure 5. Relationship of feeding rhythm with haemolymph trehalose levels in fifth instar larva and prepupa of *A. janata* in LD 12:12 ($p^{*} < 0.01$).

may account for the higher trehalose levels in *A. janata*. When feeding is high, FB is geared under optimal nutritional conditions to synthesize trehalose at some maximal rate (Friedman, 1967) and the brake in this system appears when end point inhibition of trehalose occurs (Murphy and Wyatt, 1965). The increased food consumption during the final instar may be an adaptation to tide over energy requirements in the successive non feeding pupal stage (Waldbauer, 1968). Consumption and utilisation of dietary constituents of Castor (*Ricinus communis*) leaves by semilooper caterpillar *A. janata* were reported by Ramdev and Rao (Ramdev and Rao, 1979). Thus blood trehalose levels respond strikingly to the nutritional state (Hills and Golds worthy, 1968) ie. quantity and quality of food intake (Hansen, 1964), to the developmental stage and to physiological conditions (Howden and Kilby, 1960) of the insect.

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Morphological variations in Natural Population of *Culex vishnui* Complex

N. Tandon, S. Bhattacharya, I. Sen, B. Basak
Department of Medical Entomology, Calcutta School of Tropical Medicine
Calcutta - 700 073 and

S. K. Tandon
Zoological Survey of India, New Alipore, Calcutta - 700 053.

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Abstract: Of a total of 581 mosquitoes belonging to Culex vishnui complex, collected from human dwelling (HD) and cattle sheds (CS) in randomly selected villages of district Hooghly and South 24- Paraganas, West Bengal, India, 58 (10%) specimens (*Cx, tritaeniorhynchus* 48.2%, *Cx. vishnui* 41.3% and *Cx. pseudovishnui* 10.3%) showed morphological variations with respect to the length and type of the pale band on the proboscis and did not conform to the description of the type specimens of the respective species. *Cx. tritaeniorhynchus* Giles 1901 and *Cx. vishnui* Theobald 1901, showed three and *Cx. pseudo vishnui* Colless 1957 two types of morphological variations. The morphological characters of the hind femur (with respect to the type and length of the dark stripe) in specimens showing variations in the proboscis, however, were in total conformity with the description of the type specimens. Correct identification of each of the three species of *Culex vishnui* complex is necessary in view of their importance in the epidemiology of the viral disease Japanese encephalitis.

INTRODUCTION

Morphological variations or abnormalities from the type form in the egg, larval and adult stages of Culex and Anapheles mosquitoes have been reported (Colless, 1957, Wattal *et al.*, 1969, Nagpal and Sharma, 1983, Nagpal, 1990, Gunasekaran *et al.*, 1990). Colless (1957), during systematic studies of the genus Culex in Singapore observed that despite marked resemblances between the adults of these species, their larvae showed specific differences. He further observed that the Malayan forms of *Culex vishnui* did not resemble the type specimens of the species in British Museum, nor with the original description. Reuben (1969). in an elaborate taxonomic study conducted in North Arcot district (Tamil Nadu) and Chitoor district (Andhra Pradesh), has vividly described the taxonomic characters of the three species of *Culex vishnui* complex. It was noted that the morphological characters used in identification of different specimens of three species of *Culex vishnui* complex were not always consistent.

While conducting a survey of the mosquito fauna of district 24-Parganas and Hooghly, West Bengal, 58 specimens belonging to *Cx. vishnui* complex, on close examination revealed that the length and type of the pale band on the proboscis of the three members (i.e *Cx. vishnui, Cx. pseudovishnui* and *Cx. tritaeniorhynchus)* constituting *Cx. vishnui* complex did not conform to the original description of the proboscis of the respective species.

In view of the importance of the members of *Cx. vishnui* complex in the epidemiology of Japanese encephalitis, (Haiti, 1976, Chakraborty, S. K. *et al.*,1977, Chakraborty, M. S. *et al.* 1981) it was considered worthwhile to enumerate and describe the morphological variations encountered in some specimens of the three species of the complex. The details are presented in this communication.

MATERIALS AND METHOD

Adult *Culex* mosquitoes were collected from human dwellings (HD) and cattle sheds (CS) from randomly selected villages in District Hooghly and South 24-Paraganas by trained insect collectors. in the early hours of the morning, from 07.00 hours to 09.00 hours with the help of test tubes, torches and aspirators and identified up to the specific level.

RESULTS

A total of 581 mosquitoes (all female) belonging to *Culex vishnui* complex [*Cx. tritaeniorhynchus*-378 (65.06%), *Cx. vishnui* 98 (16.86%) and *Cx. pseudovishnui*-105 (18.07%] were collected, of which, 58 (9.98%) specimens [*Cx.tritaeniorhynchus*-28 (48.27%), *Cx. vishnui*-24 (41.38%) and *Cx. pseudovishnui*-6 (10.34%) though apparently identical with the description of the type specimens of the respective species, exhibited morphological variations with respect to the length and type of the pale band on the proboscis and did not conform to the description of the type specimens. The morphological characters of the remaining 523 (90.02%) specimens were in total accordance with those described for the type specimens (Barraud 1934, Reuben 1969) of each of the three species of *Cx. vishnui* complex.

The data revealed that *Cx. vishnui* Theobald 1901 (Fig. A. 1, 2, 3) and *Cx. tritaeniorhynchus* Giles 1901 (Fig. A. 6, 7, 8) showed three and *Cx. pseudovishnui* colless 1957, (Fig. A. 4, 5) two types of morphological variations in the banding pattern of the proboscis. Detailed description of the variations encountered in the members of *Cx. vishnui* complex are presented in Table 1. A brief account of the characters of the proboscis of the type specimens of each of the three species of the complex have also been provided for comparison. of the *24 Cx. vishnui* Theobald 1901, showing morphological variations, 7 (29.16%) were collected from cattle sheds (CS) and 17 (70.83%) from human dwellings (HD), whereas of the 28 *Cx. tritaeniorhynchus*, Giles 1901, 4 (14.28%) specimens were from cattle shed (CS) and 24 (85.71%) from human dwellings (HD). On the other hand, only, six specimens of *Cx. pseudovishnui* Colless 1957, of which 2 (33.33%), were from cattle sheds and 4 (66.66%) from human dwellings (HD) showed morphological variations in the proboscis.

It is worthwhile mentioning that in specimens showing variation in the proboscis, the characteristic features on the hind legs (i. e. with respect to the type and length of the dark band on the femur) were in total accordance with the descriptions of the type specimens of the respective species (Barraud 1934, Reuben 1969).

DISCUSSION

Morphological variations in the form of ornamentation of palpi, wings and tarsi of hind legs including melanic morphometric variations (Dash *et al.*, , 1983, Nagpal and Sharma 1983, Nagpal, 1990, Gunasekaran *et al.*, 1990) have been frequently recorded in Anophelines. Perusal of literature reveals thst such variations, though of infrequent occurrence in Culex species have been observed in mosquitoes belonging to the *Culex vishnui* complex (Reuben, 1969).

Colless (1957), while conducting systematic studies of the genus Culex in Singapore observed the presence of at least four distinct species, all identifiable in the adult stages as *Cx. vishnui*. On comparison, these forms did not show any resemblance to the type specimens, which in turn differed markedly from any existing description, including the original by Theobald (1901) who observed that the true *Cx. vishnui* though a widespread species is rarely encountered, and even more rarely recognised.

Reuben (1969) in her investigations on mosquitoes collected from predominantly rural areas of North Arcot District (Tamilnadu) and Chitoor District (Andhra Pradesh), observed that some specimens of *Cx. vishnui* were heavily speckled on the legs, proboscis and wings and resembled *Cx. sitiens*,. as also observed by Colless (1957). She further noticed that a small proportion of *Cx. pseudovishnui* resemble *Cx. vishnui* and the likelihood of their being misidentified as *Cx. vishnui* can not be overruled.

During the present study the proboscis of 10% of the mosquitoes belonging to *Cx. vishnui* complex, collected from district Hooghly and 24-Parganas, West Bengal, showed variation with respect to the type and length of the pale band on the proboscis, and the characters did not conform to those described for the proboscis of the type specimens of each the three

Table 1. Morphological variations in *Culex vishnui* complex, from district Hooghly and 24-Parganas, West Bengal, India.

Culex vishnui Theobald 1901

Locality	No. of specimens	Habitat		Nature of variation
		Cattle shed	Human dwelling	
Kakdwip	2	1	1	Tip of proboscis pale; dark band runs medially throghout the entire length of the proboscis. Fig. A.1.
Sonarpur	1		1	
Kulpi	3	2	1	
Thakurpukur	3		3	
Naihati	2		2	
Thakurpukur	2	1	1	Apical 1/3 of proboscis dark; with a narrow pale strip medially; the dark area extending laterally on one side upto the base. Fig. A.2.
Kulpi	1	..	1	
Baruipur	3	1	2	
Hooghly	2		2	
Naihati	1		1	
Naihati	2	1	1	Apical 1/3 of proboscis dark, gradually fading towards the middle, basal 1/2 of the length pale. Fig. A.3.
Joynagar	2	1	1	
Total	24	7	17	

Description of proboscis of type specimens [as per-Reuben, R. 1969 Bull. Ent. Res. 1969. vol.58. 643-652].–
Proboscis dark, with a pale band covering 1/4 to 1/6 usually 1/5 of its length and extending basally a little past the midpoint. Pale scales frequently scattered over dark areas, usually light to heavily speckled on the basal part of the proboscis, and less frequently on the apical part as well; rarely the pale scaling forms an almost continuous pale area on the underside of the proboscis, extending from the pale band nearly to the base.

Culex pseudovishnui Colless 1957

Locality	No. of specimens	Habitat		Nature of variation
		Cattle shed	Human dwelling	
Joynagar	1	1		1/3 length or little more of apex of proboscis dark, remaining length entirely pale. Fig. A.4.
Kakdwip	3	1	2	
Sagar	1		1	Apcial 1/3 of the proboscis dark, the dark area extending laterally on one side towards the base. Fig. A.5
Kulpi	1		1	
Total	6	2	4	

Description of proboscis of type specimens [as per Reuben R. 1969 Bull Ent Res. 58:643-652].–
Width of pale band 1/4 to 1/6 length of proboscis, pale scaling sometimes present on dark area of proboscis basal to the pale band, these scales usually confluent and never producing a speckled effect as in *Cx. vishnui*, pale scales on basal part of proboscis occasionally few, and a frequently not present on this area.

Culex tritaeniorhynchus Giles 1901

Locality	No. of specimens	Habitat		Nature of variation
		Cattle shed	Human dwelling	
Naihati	2	..	2	Proboscis uniformly dark, except at the base and apex. Fig. A.6.
Kulipi	3	1	2	
Hooghly	1		1	
Thakurpukur	1		1	
Kakdwip	2		2	
Joynagar	2	.	2	
Sonarpur	2	1	1	
Thakurpukur	2	1	1	Tip of the proboscis pale, followng 1/5 length dark, with well defined speckling on lateral sides throughout the remaining length. Fig. A.7.
Sagar	3		3	
Kulpi	4	..	4	
Hooghly	1	..	1	
Diamond Harbour	1	1	.	
Thakurpukur	2	..	2	More than apical 1/2 of length of proboscis uniformly dark. Basal half pale. Fig. A.8.
Baruipur	2	..	2	
Total	28	4	24	

Description of the proboscis of type specimens [as per Reuben, R. 1969. Bull. Ent. Res. 58: 643-652].–
Dark areas of the proboscis with more as less extensive pale patches especially ventrally on the basal part, pale scaling infrequently absent, this region appearing entirely cream coloured. Apical part of proboscis also occasionally with pale scaling.

species of the complex (Fig. A).

Analysis of data revealed that of 98 *Cx. vishnui* collected, 24 (7 from cattle sheds and 17 from human dwellings) exhibited three types of variation in the colour pattern of the proboscis (Table 1, Fig. A) and did not conform to Reuben's (1969) description, who observed that the length of the pale band on the otherwise dark proboscis of *Cx. vishnui* mosquitoes varied between one quarter to one sixth, usually one fifth length and extended basally a little past the midpoint.

Similarly, of 378 (*Cx. tritaeniorhynchus* collected during our study, 28 (4 from cattle sheds and 24 from human dwellings) showed three types of variation in the banding pattern of the proboscis (Table 1, Fig. A) and differed considerably from what is described for specimens collected from South India (Reuben, 1969).

Amongst 105 *Cx. pseudovishnui* collected during the survey the proboscis of six specimens (2 from cattle sheds and 4 from human dwellings) exhibited two types of variation (Table 1, Fig. A) and showed no resemblance to the characters already described (Reuben 1969), where the width of pale scales on the proboscis varied from a quarter to 1/6 length of the proboscis.

Perusal of literature reveals that the confusion with respect to the taxonomic status of *Cx. vishnui* complex was finally settled due to the efforts of Reuben (1969). Repeated isolations of JE virus from *Cx. vishnui* in West Bengal (Chakraborty, S. K. *et al.*, 1975, 1977, Chakraborty, M. S. 1980) and from *Cx. pseudovishnui* and *Cx. tritaeniorhynchus* in different parts of the country (Hati, 1976; Chakraborty, M. S. 1981) emphasize the need for correct identification of the species involved in the transmission of the disease, and does not allow for any lacunae in the field. That morphological variations are due to difference in climate, topogr-

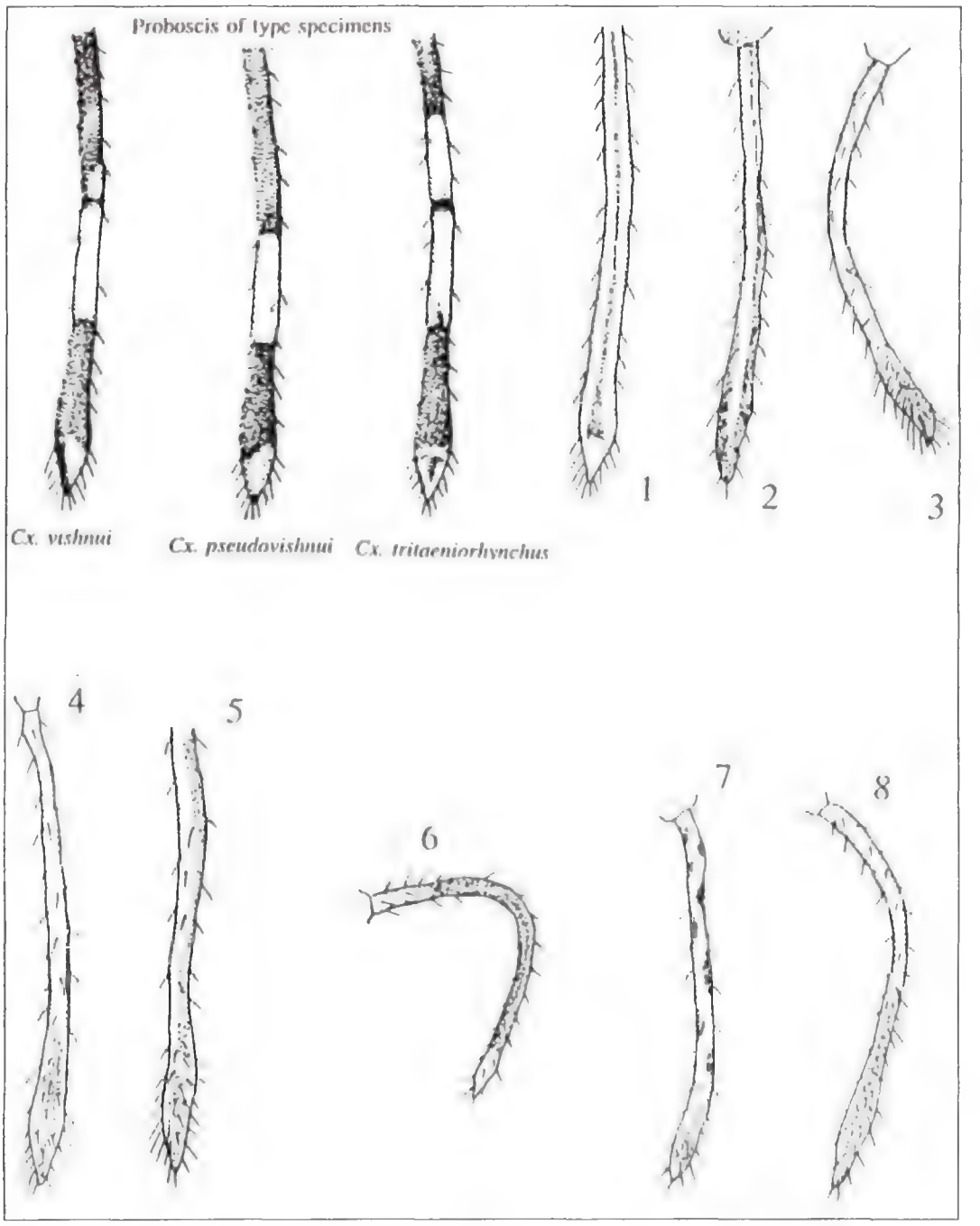

Fig. A. Morphological variations in the proboscis of *Culex vishnui* complex
Fig. 1, 2, 3. Variations in *Culex vishnui* Theobald 1901.
Fig. 4,5. Variations in *Culex pseudovishnui* Colless 1957.
Fig. 6, 7, 8. Variations in *Culex tritaeniorhynchus* Giles 1901.

aphy and season (Dash *et al.*, 1983, Gunasekaran *et al.*, 1990) and affect the behaviour and vectorial capacity of the specimens (Dash *et al.*, 1983), needs to be determined.

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Lipase Activity During Metamorphosis of *Chrysomyia rufifacies*

J. J. Pol and V. A Sawant
Department of Zoology, Shivaji University
Kolhapur, 416 004, Maharashtra State
India

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Abstract: The changes in lipase activity during metamorphosis of *Chrysomyia rufifacies* have been studied. The lipase activity was maximal at broad pH range 8.5 to 9.0. The lipase mainly hydrolyzed the triglycerides to diglycerides and fatty acids. There is increase in the lipase activity from prepupa to second day pharate adult and decrease from second day pharate adult to fifth day pharate adult. The maximum activity was observed in second day pharate adult. The physiological role of the lipase during metamorphosis is discussed.

Key words: Lipase, Metamorphosis, Blowfly, *Chrysomyia rufifacies*.

INTRODUCTION

Insect pupae and their significance are considered by Hinton (1946, 1948, 1963), the morphological aspects of metamorphosis by Snodgrass (1954), the biochemical aspects by Agrell and Lundquist (1973), Thomson (1975) and Sridhara (1981), and Physiological aspects by Wigglesworth (1964), Gehring and Norhiger (1973) reviewed the development of imaginal discs in *Drosophilia*. Presently lipase activity during embryogenesis and larval growth of *Chrysomyia rufifacies*. has been studied (Pol and Sawant 1989, 1990). A few studies have been carried out on the activity of lipase in insect metamorphosis *Trogoderma granarium* (Nandan *et al.*, 1973). Fat is the chief nutrient form in which energy is stored. It is usually present in greatest amounts in the mature larva before metamorphosis. Lipids are utilised for providing energy for metamorphosis (Rao and Agarwal 1970, 1971). The lipid content gradually decreases to about 41% of the dry weight in the pupa and triglycerides account for about 90% of these lipids (Rao and Agarwal 1970). For utilization of lipids, especially triglycerides and for energy production, the component of fatty acids must be hydrolyzed. Lipases are enzymes which are responsible for such hydrolysis. During metamorphosis of insects extensive histolysis and histogenesis occur. In most of the insect species, the energy required for the transformation is derived from lipid, mainly reserves of triacylglycerols and the lipase activity is instrumental in the release of energy (Agrell and Lundquist 1973, Nandan *et al.*, 1973). Therefore the present study attempts to provide information on the lipase activity during metamorphosis of *Chrysomyia rufifacies*.

MATERIALS AND METHODS

The insects were taken form our permanent cultures maintained in the laboratory under constant conditions of temperature and humidity. The method of rearing the blowflies as described by Munich (1959) was followed.

The hatching of eggs occurred within 20 hours after oviposition. The larval growth was computed from the mean time of egg hatching (± 1.0 Hr.) to the prepupal stage. The larval stage lasted for four days. The prepupation and pupation lasted for one and five days respectively. Studies were carried out at an interval of 24 hours in each case of prepupa (pp), Pharate adults (P1, P2, P3, P4, P5) and adults (A1, A2..).

For the study of lipids and lipolytic activity, the following stages were selected: Prepupae (pp), first day pharate adult to fifth day pharate

adult developmental stages (P1, P2, P3, P4, P5), first day adults and second day adults. (A1, A2).

All the solvents were of reagent grade and were obtained from E. Merck and Co. Rahway, N. J., U. S. A. and B. D. H., England. Unless otherwise indicated solvents were redistilled in the laboratory under anhydrous condition before use. Diphenyl Carbazide was purchased from E. Merck, Dermstat, Germany. Diphenyl carbazone was of Veb. Jenapharm Laborchemie, Apolda, Germany. Triolein, Diolein, Monolein, Stearic Acid, BSA were obtained from Sigma Chemical company, U. S. A.

The prepupae (pp), pharate adults (P1, P2, P3, P4, P5) and adults (A1, A2) were isolated, cleaned with distilled water weighed and homogenized in cold double distilled water using a ground glass pestle and mortar. The homogenates were diluted with cold double distilled water so as to get 1% (wt/vol) concentration. Such homogenates were used for the biochemical assay of the lipolytic activity (EC 3.1.1.3.) as a described by Patil *et al.*, (1983).

Protein was determined by the method of Lowry *et al.*, (1951). The emulsion of olive oil was prepared according to the method adopted by Patil *et al.*, (1983).

Triglycerides from olive oil were purified using neutral alumina gel. Purity of triglycerides from olive oil was checked by thin layer chromatography. Extraction and purification of lipids was carried out using Folch's improved method (1957). Neutral lipids were separated by thin layer chromatography on silica gel coated plated (20 x 20 cm) prepared according to Wagner *et al.*, (1961).

Results

Changes in lipase activity during metamorphosis are shown in Fig. 1. There was a gradual increase in lipase activity from prepupa to first day pharate adult and rapid increase from first day pharate adult to second day pharate adult. The maximum activity was observed in second day pharate adult. A sharp decline in lipase activity was observed from second day pharate adult to third day pharate adult and then slow decrease from third day pharate adult to fifth day pharate adult and first day adults. The minimum activity was observed in freshly emerged adult. The lipase activity was maximal at the broad pH range 8.5 to 9.0. The hydrolysis products of triacylglycerol by lipase on thin layer chromatography plates were diacylglycerol and fatty acids.

Discussion

The hydrolysis of triacylglycerol clearly showed that lipase is present in the pupal homogenates of *Chrysomyia rufifacies*. Similar observations had been reported in the pupal homogenates of *Trogoderma granarium* by Nandan *et al.*, (1973).

The broad optimum pH range 8.5 to 9.0 of the enzyme activity in the second day pharate adult homogenate indicates the presence of alkaline lipase. Working with khapra beetle, *Trogoderma granarium*, Nandan *et al.*, (1973) found that the lipase activity of a homogenate of whole pupa or pharate adult was optimal at pH 7.6.

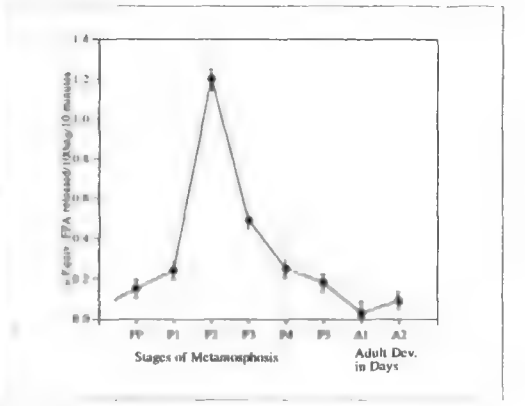

Fig. 1. Lipase activity during metamosphosis of *Chrysomyia refifacies*.

The increase in lipase activity from the prepupa to first day pharate adult indicates the beginning of histolysis. The rapid increase in the lipase activity from first day pharate adult to the second day pharate adult clearly shows the active role of lipase in the histolysis which provides energy for metamorphosis and materials for histogenesis. In the second day pharate

adult no larval organs were observed. The maximum activity observed was in the second day pharate adult suggest the extensive histolysis requiring energy and this energy is derived from the breakdown of lipids.

The decrease in lipase activity from second day pharate to fifth day pharate adult may be due to histogenesis. Thus the main source of energy during metamorphosis of *Chrysomyia rufifacies*. is lipid and the lipolytic activity is instrumental in the release of energy. Similar observations were reported by Nandan *et al.*, (1973) and Agrell and Lundquist (1973).

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Studies on the Natural Enemies of the Wax Scale *Drepanococcus chiton* (Green) on Ber and Guva

M. Mani

Biological Control Laboraroty I, Division of Entomology and Nematology
Indian Institute of Horticultural Research, Bangalore - 560 089, India

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Abstract: The scale insect, *Drepanococcus* (=*Ceroplastodes*) *chiton* (Green) caused severe damage to ber and guava around Bangalore. The survey for its natural enemies in 1990 and 1991 yielded six primary parasitoids, viz. *Anicetus ceylonensis* Howard, *Diversinervus elegans* silvestri, *Metaphycus* sp. A. nr. *helvolus* (compere), *M.* sp. B. nr. *helvolus*, *Philosindia* sp. nr. *longicornis* Noyes and Hayat (all encyritids) and *Cephaleta brunniventris* Motsch (Pteromalidae) and four cocinellid predators namely; *Chilocors nigrita* Fabricius, *Cryptolaemus montrouzieri* Mulsant, *Menochilus sexmaculata* (Fabricius) and *Scymnus* sp. Some of them were recorded for the first time on *D. chiton* in India. The results on the impact of the indigenous natural enemies strongly indicated that the parasitoids acted predominantly on *D. chiton* as compared with predators. Only on guava, *C. nigrita* exerted some influence on the scale Insect. Otherwise, the parasitoids, chiefly *A. ceylonensis* and *C. brunniventris*, were mainly responsible for the suppression of *D. chiton* both on ber and guava.

Key words: Scale insect, *Drepanococcus chiton*, parasitoids, predators, ber, guava.

INTRODUCTION

The wax scale *Drepanococcus (= Ceroplastodes) chiton* (Green) is a polyphagous pest recorded on ber *(Zizyphus mauritiana* Linnaeus) in Pakistan (Ghani & Muzaffar, 1974). Even though the scale insect was described as early as 1908 (Green, 1908), little work has been done so far except the record of two parasitoids on *D. chiton* in Pakistan. Since the insect has a waxy covering, chemical pesticides could not suppress the scale population adequately (Patel *et al.*, 1991). In fact, the scale problem was more pronounced after the use of synthetic insecticides which had disturbed natural enemy complex of the scale insects responsible to keep the scales under check (Argyriou & Debach, 1968). In 1990, *D. chiton* appeared in severe form on ber and guava trees around Bangalore. The present investigation was carried out to identify the natural enemies and also to study their impact in the suppression of the wax scale on ber and guava.

MATERIAL AND METHODS

(i) **Survey**:

Surveys were carried out during 1990 and 1991, to determine the natural enemies of *D. chiton* Homoptera: coccidae on ber and guava in Bangalore and Kolar districts of Karnataka state. In addition to the collection of natural enemies directly from the field, scale infested shoots were brought to the laboratory in cloth bags and kept in wooden cages (30 x 30 x 30 cm). Each cage was provided with cloth walls on three sides and a class front. The parasitoids and predators that emerged were collected and preserved. The scale and its natural enemies were sent to International Institute of Entomology, London and also to Dr. M. Hayat, Aligarh Muslim University for identification.

(ii) ***Impact of natural enemies***

(a) *Selection of Orchards*

Ber: A four year old ber orchard (variety: Umran) infested with *D. chiton* was chosen at

Table 1. List of natural enemies of *D. chiton* collected in ber and guava orchards during 1990-91.

Natural enemy	Family	Order
Primary Pararsitoids		
Anicetus ceylonensis Howard	Encyrtide	Hymenoptera
Diversinervus elegans Silvestri	"	"
Metaphycus sp. A. nr. *helvolus* Compere	"	"
Philosindia sp. *M.* sp. B. nr. *heliocus*	"	"
Longicornis Noyes and Hayat	"	"
Cephaleta brunniventris Motsch	Pteromalidae	"
Predators		
Chilocorus nigrita Fabricius	Coccinellideae	Coleoptera
Cryptolaemus montrouzieri Mulsant	"	"
Menochilus sexmaculata Fabricius	"	"
Scymnus sp.	"	"
Hyperparasitoids		
Marietta leopardina Motsch	Aphelinidae	Hymenoptera
Promusidea unfaciativentris Girault	"	"
Cheiloneurus sr. nr. *cyanonotus* Waterson	Encyrtidae	"
Aprostocetus purpureus Cameron	Eulopidae	"

Block No. 3, Indian Institute of Horticultural Research Farm, Hessaraghatta, Bangalore. The scale appeared by the end of April 1990; applications of quinalphos (0.05%) did not reduce its infestation. Therefore, sampling was initiated from June and continued upto January '92 at monthly intervals.

Guva: A guava garden (Variety: Allahabad Safeda) of five year old infested with the scale insect was selected in a farmers' field at Doddaballapur situated 32 km from Bangalore. Regular application of insecticides like monocrotophos failed to control the scale in May-July '91. Sampling was done from July to November, 1991.

(b) *Sampling*

Sampling was done on five randomly selected infested trees. From each tree, five sides shoots each 30 cm long, were removed and kept in the wooden cages (30 x 30 x 30 cm) for the emergence of natural enemies. The parasitoids and predators observed directly on the plants were also added to the respective samples. After 25 days of sampling, the scale infested shoots were removed from the wooden cages and examined for parasitoid emergence holes to record the level of parasitism.

RESULTS

Survey: Samples of *D. chiton* collected during 1990-91 yielded six primary prasitoids, four hyperparasitoids and for four predators (Table 1). Among the primary parasitoids, only the encyrtid *Anicetus ceylonensis* Howard and the pteromalid *Cephaleta brunniventris* Motsch. occurred in large numbers frequently on both crops. The other four parasitoids species were uncommon. Hyperparasitoids, *Prumuscidea unfaciativentris* Girault and *Marietta leopardina* Motsch were frequently collected from scale infested schoots of guava and bar. The other two were less common. Four coccinellid predators were associated with the scale in the collections. *Chilocorus nigrita* Fabricius was frequently encountered in guava orchards, while *Cryptolaemus montrouzieri* Mulsant was observed on ber trees infested with the scale.

Impact of natural enemies: Severe scale infestation was noticed in Block No.3 of I. I. H. R.

Table 2. Population of natural enemies and per cent parasitism on *D. chiton* at I. I. H. R. Farm.

Date of sampling	* Number of natural enemies emerged and observed				Total parasitism (%)
	Predators		Parasitoids		
	Cryptolaemus	*Scymnus*	*Anicetus*	*Cephaleta*	
26-6-91	0.00	0.00	0.00	0.00	0.00
20-7-91	1.30±0.41	0.50±0.04	3.40±0.54	0.60±0.06	5.60±1.74
26-8-91	2.40±0.72	1.20±0.10	8.60±2.18	4.20±1.50	9.17±1.90
21-9-91	1.60±0.24	0.80±0.03	15.70±4.80	8.80±1.84	14.28±3.20
18-10-91	3.10±0.60	2.10±0.40	26.20±7.26	16.20±3.36	30.80±6.84
28-11-91	4.20±1.30	1.70±0.07	17.80±6.37	27.80±7.42	50.20±9.26
28-12-91	1.30±0.47	0.60±0.01	32.10±9.28	10.90±6.84	78.34±7.66
27-1-92	0.00	0.00	47.60±11.34	7.20±2.34	97.56±1.26

*Mean ± Standard deviation

Table 3. Population of natural enemies and per cent parasitism on *D. chiton* on guava at Doddaballapur.

Date of sampling	Number of natural enemies emerged and observed			Total parasitism (%)
	Parasitoids			
	Chilocorus	*Anicetus*	*Cephaleta*	
19-7-91	4.40±1.24	16.50±6.74	4.60±1.20	20.42±4.36
1-8-91	7.60±2.17	28.40±10.36	9.70±2.17	34.27±7.42
12-8-91	11.20±3.46	37.40±13.42	14.30±4.28	53.35±6.48
27-8-91	16.30±6.21	80.30±18.46	23.30±6.48	74.16±3.70
10-9-91	4.50±1.24	17.60±4.18	9.40±2.81	90.25±4.46
9-10-91	1.00±0.50	4.20±0.41	3.50±0.57	96.48±3.21
15-11-91	0.50±0.04	0.00	0.00	98.00±0.56

*Mean ± Standard deviation

Farm on ber in May 1991. Initial sampling in June did not indicate the activity of the natural enemies but predators and parasitoids were observed form July onwards. The predators *C. montrouzieri* and *Scymnus* sp., were observed feeding on the crawlers and the immature scale. The predator population remained very low throughout the experimental period. A maximum of 4.20 and 2.10 per sample of *C. montrouzieri* and *Scymnus* sp., were observed in November and October respectively. Occasionally, the larvae of *Menochilus sexmaculata* Fabricius was found feeding on the young scales but its population was negligible.

The primary parasitoids *A. ceylonensis* and *C. brunniventris* were found emerging in large numbers than the other parasitoids. *A. ceylonensis* was dominant throughout the experimental period. It was less active in June but a peak population of 47.60 per sample as observed from the samples collected in January '92. *C. brunniventris* were also equally active against *D. chiton*. It suppressed *A. ceylonensis* in terms of the number of parasitoids emerged from the November sampling. A maximum of 27.80 parasite adult of *C. brunniventris* was collected in November '91 (Table 2). The other parasitoids like *Metaphycus helvolus* sp. n. and *Philosindia longicornis* sp. n. also emerged in very small numbers.

The parasitism rate mostly due to *A. ceylonensis* and *C. brunniventris* was initially low in June '91. The increase in percentage of parasitism was progressive and a peak of 97.56 per cent parasitism was recorded in January '92. Incidentally, sampling done in January also

yielded large number of parasitoids. Fresh scale infestation was not noticed in the subsequent months even upto December. In two other Orchards also at I. I. H. R. Farm, where regular observations were not taken, *D. chiton* was noticed in severe form in 1990 on some ber trees and the parasitoids were collected in large numbers in the same year. The scale disappeared subsequently and never appeared again in 1991 and 1992.

Guava: The infestation of *D. chiton* was first detected in severe form in July' 91 on guava at Doddaballapur. The initial sampling revealed the natural parasitization and predation of local biotic agents. The coccinellid *Chilocorus nigrita* Fabricius was seen surprisingly in considerable numbers throughout the experimental period. No other predator was observed. A maximum population 16.30 of *C. nigrita* per sampling was noticed by the end of August, 1991. It might have had considerable influence on the scale insect (Table 2).

As in the case of ber, here also both *A. ceylonensis* and *C. brunniventris* were found to emerge in larger numbers than the other primary parasitoids. They contributed significantly in reducing the scale population in the subsequent months. As many as 80 and 23 adults of *A. ceylonensis* and *C. brunniventris* emerged in the samples collected on 27th August.

Initial percentage parasitism chiefly due to *A. ceylonensis* and *C. brunniventris* was moderate and peaked by middle of November '91. In some of the affected shoots, all the scales were found parasitized. In the subsequent year up to December '92, fresh scale infestation never appeared and the orchard was completely free from *D. chiton*. The same sequence occurred in yet another guava orchard at chintamani (90 Km from Bangalore); although observations were not made, a large number of natural enemies were collected in 1990.

DISCUSSION

The present record of *D. chiton* appeared to be new on ber in India and on guava elsewhere also. Though the pest was reported in about 10 countries, its natural enemies were known only from Pakistan (Ali, 1975; Ghani & Muzzafar 1974). All the six primary parasitoids recorded in the present investigations appeared to new on *D. chiton* in India eventhough some of them were reported earlier on other species on coccids (Hayat, 1986; Farooqi & Subba Rao, 1986). For predators observed in the present study were general predators of homopteran insects reported in various countries, though not on *D. chiton*. Among the hyperparasites, the encyrtid *Cheiloneurus* sp. n. *Cyanonotus* appeared to be new record in India.

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Development and Differentiation of Male Reproductive Organs In *Opisina arenosella* Walker.

P. B. Santhosh Babu
Regional Research Laboratory (CSIR)
Industrial Estate P. O., Trivandrum 695 019, India

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Abstract: Development of the reproductive system in *Opisina arenosella* is traced from 1st instar to differentiation of the definitive organs. The testis becomes distinct in the Ist instar larva. Morphological changes of testis including volume, tracheation, fusion and torsion are observed. Spermatogenesis during ontogeny is studied using light microscope and phase contrast microscope. Eupyrene sperm bundles are observed in the late larval stage, while the apyrene sperm bundles appear only in the early pupal period onwards. The male reproductive tract undergoes substantial changes from last instar larva to 4-day pupa. The genital cord, the only reproductive tract in larva connects the testis to the Herold's organ, an ectodermal inpushing in the 9th abdominal segment of the larva. Herold's organ give rises to cuticle lined ejaculatory duct and aedeagus. The genital cord differentiates into upper and lower vasa deferentia and seminal vesicle. The distal end of the genital cord, ampulla produces accessory reproductive glands. Rudiments of accessory reproductive glands first appear in prepupa. All organs are fully differentiated in 4-day pupa.

Key words: *Opisina arenosella* Walker, testicular development, spermatogenesis, differentiation of genital cord and Herold's organ.

INTRODUCTION

Different aspects of male reproductive system have been worked out in a number of lepidopteran insects. Postembryonic development of the system has been described in *Ostrinia nubilalis* (Jones *et al.*, 1984). Reports are available on the morphology of the reproductive tract in mature larva, pupa and adult in *Manduca sexta* (Reinecke *et al.*, 1983), on morphology in *Plathypena scabra* (Bunting & Perigo, 1983), on structure, development and physiology in *Phthorimaea operculella* (Brits, 1978 & 1979). Testicular development and spermatogenesis have been studied in many lepidopteran insects (Numata & Hidaka, 1980; Lai-Fook, 1982; Scheepens & Wysoki, 1985; Sridevi *et al.*, 1989). The present investigation deals with the spermatogenesis and development and differentiation of reproductive tract in black-headed caterpillar, *Opisina arenosella*, which revealed certain special features.

MATERIALS AND METHODS

The required insects were taken from the colony maintained in the laboratory as described earlier (Santhosh Babu and Prabhu, 1987). Testes of insects from various stages of development, Herold's organ and genital cord from VI-VIII instar larvae and prepupa and accessory reproductive organs from pupae and adult were dissected out in insect saline. The materials which were traced with the aid of a camera lucida were examined under a binocular dissection microscope.

For histological studies, testis and associated structures were fixed in aqueous Bouin's fluid. Paraffin sections were cut at 6μm, stained either with Ehrlich's haematoxylin eosin, or with Mallory's triple stain in the routine histological manner. Phase contrast microscope was used for teased preparation of testis from various stages of development. Measurements were taken using an ocular micrometer. The volume of larval testis was calculated using the formula $V=\pi/6$ (length x width)^2 (Nishiitsuji-Uwo, 1959) and testis volume in prepupa, pupa and adult was determined by the formula $V=4/3\pi ab^2$ (Loeb *et al.*, 1984) where 'a' and 'b'

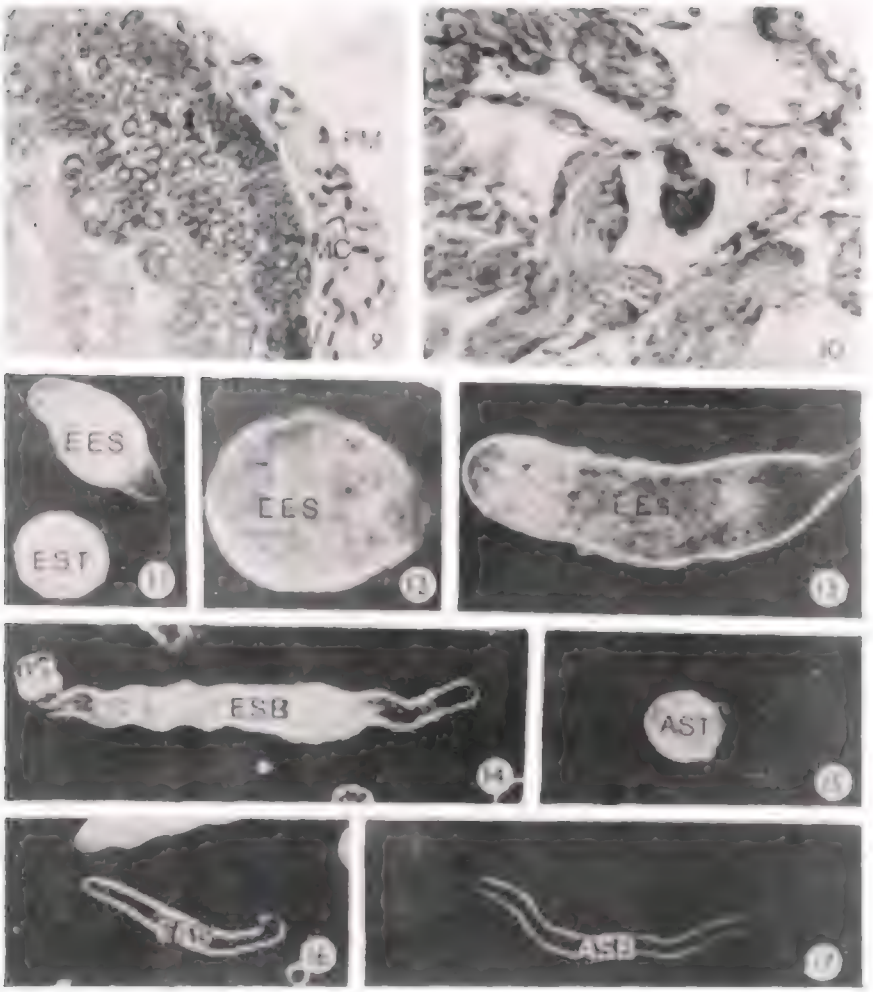
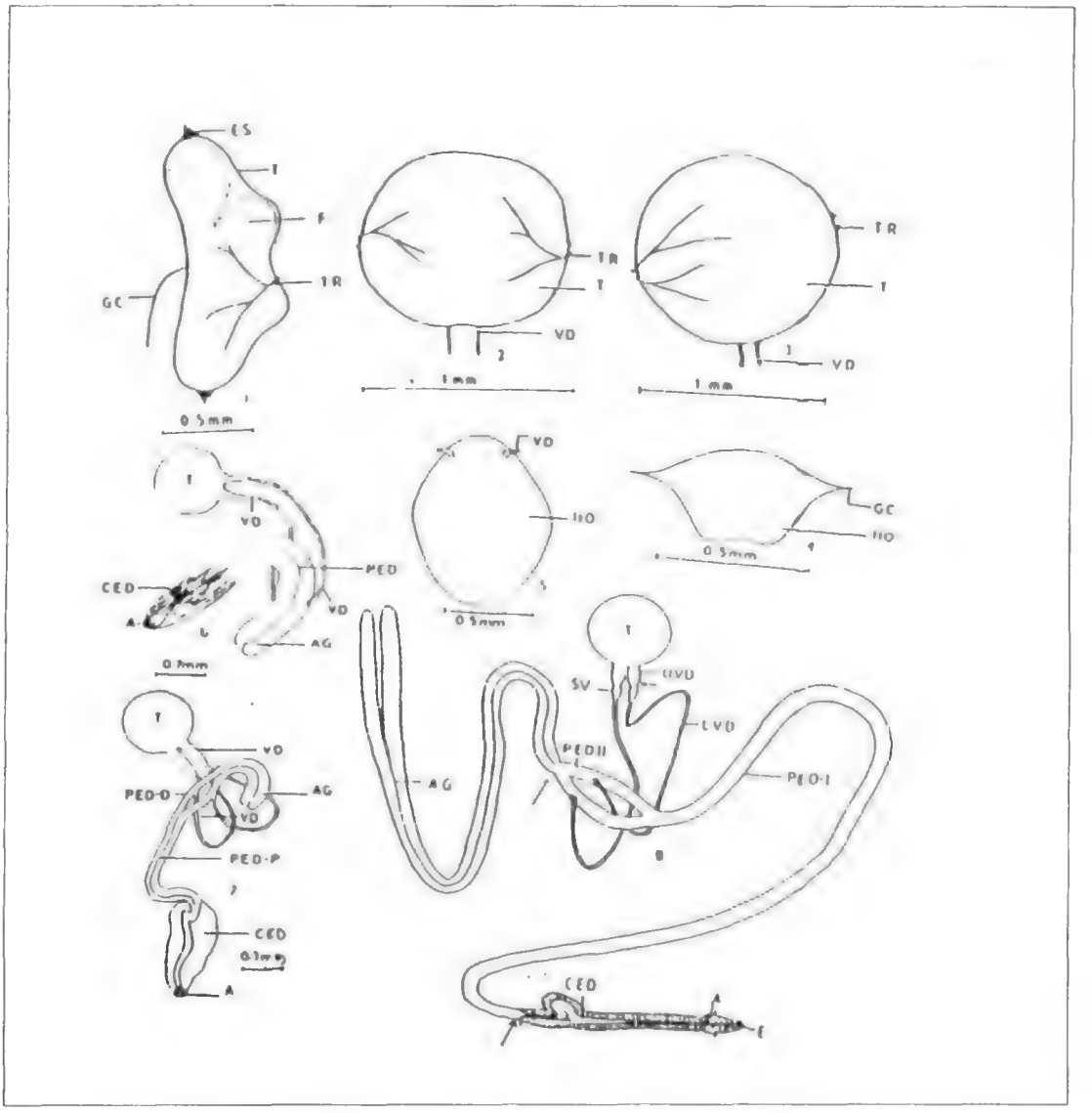


Fig. 1. Testis of VI instar larva, dorsal right view; Fig.2 Fused testis of prepupa showing the left and right tracheae, dorsal view. Fig. 3. Fused, spherical testis of 6-day pupa showing the torsion of the testis evidenced by the twisting of the tracheae, dorsal view. Fig.4 Herold's organ of VII instar larva. Fig.5 Herold's organ of prepupa. Fig.6 Reproductive organs in 0-day pupa. Fig.7 Reproductive organs in 1-day pupa. Fig.8 Diagrammatic representation of the fully differentiated internal reproductive organs in 4-day pupa; *constriction. Fig.9 Transverse section of the testis showing the membrana communis and peritoneal membrane of the testis x 100. Fig.10 Transverse section of the testicular follicle showing the follicular x 400. Figs.11-17 Unstained phase contrast photomicrographs showing the sequence of maturation process of eupyrene and apyrene spermatid cysts. Fig. 11. Eupyrene spermatid cyst (Spherical shape) and elongating eupyrene spermatid cyst x 400. Fig.12-13. Elongating eupyrene spermatid cyst x 400. Fig.14 Eupyrene sperm bundle x 200. Fig.15 Apyrene spermatid cyst (spherical shape) x 200. Fig.16 Elongating apyrene spermatid cyst (spindle shape) x 200. Fig.17 Apyrene sperm bundle x 200. **Legends to figures**. A, Aedeagus; Ag, Accessory gland; ASB, Apyrene sperm bundle; AST, Apyrene spermatid cyst; CED, Cuticle lined ejaculatory duct; CL, Capsula lobuli; E, Endophallus; EAS, Elongating apyrene spermatid cyst; EES, Elongating eupyrene spermatid cyst; EST, Eupyrene spermatid cyst; F, Follicle; GC, Genital cord; HO, Herold's organ; LVD, Lower vas deferens; MC, Membrana communis; PED, primary ejaculatory duct; PED-D, Primary ejaculatory duct distal part; PED-P, Primary ejaculatory duct proximal part; PED-I Primary ejaculatory duct unpaired; PED-II, Primary ejaculatory duct paired; PM, Peritoneal membrane; SV, Seminal vesicle; T, Testis; TI, Tunica interna; TR, Tracheae; UVD, Upper vas deferens; VD, Vas Deferens.



were the radius of the long and short diameter respectively.

Results

Gross anatomy of testis: The testes are distinctly paired attached to the body wall by epithelial strands on each side of middorsal line in the 5th abdominal segment in larval stages. Each testis is made up of four follicles with narrow proximal and broader distal region (Fig. 1). Two pair of trachea, left and right also attach to the testis. During the prepupal stage, the paired testes move towards the dorsomedian line and fuse along the mid-dorsal line forming a single more or less spherical structure (Fig. 2). Now the epithelial strands attached to the testis undergo degeneration. Torsion of the testis takes place in 6-day pupa and the tracheal branches from the left and right spiracles cross the testis to the opposite sides (Fig. 3).

The testis is enclosed in a peritoneal membrane. Within the testis the four follicles are

Table 1. Testis size and volume in different stadia of *O. arenosella* Walker (Mean of eight insects)

Insect Stadium		Age (day)	Paired testis		
			Length (mm) Mean±SD	Width (mm) Mean ± SD	Volume of testis (mm^3) Mean ± SD
Larval instar	I	-	0.024±0.002	0.017±0.002	$4.33x10^{-8}±1.8x10^{-8}$
	II	-	0.056±0.002	0.037±0.002	$2.27x10^{-6}±2.16x10^{-7}$
	III	-	0.190±0.037	0.055±0.011	$5.27x10^{-5}±4.13x10^{-6}$
	IV	-	0.250±0.010	0.070±0.008	$1.60x10^{-4}±2.75x10^{-5}$
	V	-	0.341±0.012	0.100±0.007	$6.13x10^{-4}±5.20x10^{-5}$
	VI	-	0.446±0.015	0.167±0.011	$1.83x10^{-3}±2.09x10^{-4}$
	VII	-	0.618±0.015	0.208±0.008	$8.61x10^{-3}±7.19x10^{-4}$
	VIII	-	0.758±0.020	0.400±0.022	$0.0482±7.08x10^{-3}$
Insect Stadium		Age (day)	Fused testis		
			Long diameter (mm)	Short diameter (mm)	Volume of fused testis (mm^3)
Prepupa		0-1	0.895±0.009	0.864±0.011	0.0355±0.011
Pupa		0	1.026±0.017	0.996±0.005	0.533±0.012
		2	1.201±0.015	1.165±0.007	0.856±0.015
		4	1.290±0.009	1.253±0.100	1.059±0.023
		6	1.158±0.008	1.136±0.010	0.783±0.016
		8	0.946±0.010	0.925±0.007	0.424±0.009
Adult		0	0.793±0.008	0.778±0.010	$0.251±8.56x10^{-3}$
		2	0.245±0.005	0.236±0.005	$7.18x10^{-3}±4.8x10^{-4}$

covered by a membrane, the *membrana communis* which is seen just beneath the peritoneal membrane (Fig. 9). The *membrana communis* is formed of irregularly arranged cells with deeply stained nuclei. Each follicle is bound externally by a membrane, the *capsula lobuli* and internally by another membrane, the *tunica interna* (Fig. 10). The *capsula lobuli* together with the *tunica interna* form the follicular septa. Each testicular follicle opens into a vas efferens. The four vasa efferentia converge at the hilum and open into a common vas deferens. Data on the dimension of the testes during different development stages are given in Table 1.

Spermatogenesis: The testis of I instar consists of only primordial germ cells. In the II instar, follicles begin to differentiate and predefinitive spermatogonia are observed. The testis consists of four distinct follicles in III instar. At one end of each follicular chamber lies a large nucleated mass of protoplasm known as the apical cells. The apical cells (9-11 μm diameter) are distinguishable by their poorly stainable cytoplasm. The cytoplasm of predefinitive spermatogonia arranged around the apical cells is connected with the apical cells. Later these predefinitive spermatogonia dissociate from the apical cells and become spherical. Their nuclei are large and spherical with little cytoplasm surrounding them. The predefinitive spermatogonia undergo mitotic divisions and give rise to definitive spermatogonia. In the IV instar, definitive spermatogonia through a series of mitotic divisions give rise to a cluster of cells which are enclosed in a cellular envelope, the cyst. The cyst contains primary spermatocytes which gradually increase in number and become closely packed within the cyst. The primary spermatocytes undergo meiotic divisions in the V instar producing secondary spermatocytes

Table 2. The length (mm) of reproductive tract in pupae of differemt age and 0-day adult in *O. arenosella* Walker (Values in mean ± SD of 8 insects/age)

Insect Stadium	Age (day)	Vas deferens and seminal vesicle	Accessory gland	Primary ejaculatory duct paired	primary ejaculatory duct un-paired	Cuticle lined ejaculatory duct and aedeagus	Total length
Pupa	0	2.19±0.25	7.94±0.39*				10.13±0.33
	1	2.31±0.24	2.31±0.24	6.88±0.33**		2.19±0.024	13.69±0.34
	2	2.44±0.30	5.06±0.39	9.44±0.30**		3.44±0.30	20.50±0.71
	3	2.69±0.24	7.56±0.39	1.81±0.24	1.81±0.24	13.31±0.24	30.81±0.96
	4	2.74±0.35	10.38±0.48	2.32±0.22	2.32±0.22	17.00±0.36	39.56±0.88
Adult	0	2.81±0.24	10.75±0.35	2.44±0.30	2.44±0.30	17.50±0.39	41.44±0.95

* Length of whole ducts, ** Length of undifferentiated primary ejaculatory duct paired and unpaired.

which are arranged along the periphery of the cyst surrounding a central cavity. Predefinitive and definitive spermatogonia are continuously divided mitotically in the apical region of the follicular chamber.

The secondary spermatocytes undergo mitotic divisions resulting in the formation of spermatid cysts in VI instar. The eupyrene spermatid cysts are first observed, which are spherical in shape immediately after the division (Fig.11) but gradually elongate during this instar (Figs. 11-13). As elongation of the eupyrene spermatid cysts continues, the nuclei orientate and arrange together at one end of the cyst. Though maturing eupyrene spermatid cysts are observed, no sperm bundles are noticed in this instar. In the penultimate instar mature eupyrene spermatozoa in intact cysts as sperm bundles are first observed in the proximal part of the follicle. The sperm bundles consists of numerous filamentous spermatozoa aligned in a parallel manner and these are surrounded by a well developed sheath forming a compact unit (Fig. 14). Apyrene spermatid cysts which are first observed in the follicle are smaller in size when compared with eupyrene spermatid cysts (Fig. 15). The apyrene spermatid cysts now gradually elongate and become spindle-shape (Fig. 16). The immature cysts are arranged near the distal and progressively more mature stages are found towards the proximal region of the follicle. The cysts in the middle region of the follicles are larger in size compared to the cysts in the distal region. The elongation of the apyrene spermatid cysts continues in last instar larva. The apyrene sperm bundles are first observed in the follicle in zero-day pupa and are smaller in size than the eupyrene sperm bundles (Fig.17).

Histology and development of reproductive tract: The genital cord is the only reproductive tract in larval stages. A group of cells differentiate from the proximal region of the follicle and form a slightly elongated structure, the genital cord in IV instar larva. The elongating genital cord passes above the gut in 5^{th} and 6^{th} abdominal segments, turn laterally, travels below the gut in 7^{th} and 8^{th} segments and it reaches in the 9^{th} segment where it fuses with Herold's organ, a cuticular inpushing into the body cavity in VII instar larva. Herold's organ is somewhat flattened in shape (Fig. 4). No lumen is now found in the genital cord.

An ampulla is formed at the distal end of the genital cord where it fuses with Herold's organ in prepupa. Now the Herold's organ is oval in shape (Fig.5). Lumen appears in the genital cord for the first time and it becomes the vas deferens. The ampullae undergo differentiation

as a result they together assume "∧" shape. The vas deferens arises from the middle region of the ampulla. At one end of the Herold's organ a cavity, the genital chamber is formed. In the late stage of prepupal period, rudiments of the aedeagus begin to differentiate from the genital chamber in the form of a small projection. The vas deferens is connected to the middle region of the ampulla. Rudiments of accessory gland begin to appear from that part of the ampulla where the two ampullae contact each other and from the remaining portion of the ampulla rudiments of primary ejaculatory duct develop.

An enlargement of the vas deferens is found at the proximal end in zero-day pupa. The genital chamber differentiates into the cuticle lined ejaculatory duct which continues with the primary ejaculatory duct. So the whole system namely accessory gland, primary ejaculatory duct, cuticle lined ejaculatory duct and aedeagus become continuous (Fig.6). A transparent membranous sheath begins to appear surrounding the cuticle lined ejaculatory duct and aedeagus. The primary ejaculatory duct and accessory gland are paired, close to each other. The vas deferens opens in the distal part of the primary ejaculatory duct near the accessory gland. In 1-day pupa the paired primary ejaculatory ducts are separated from each other at the distal end; the paired ducts of the remaining part in the proximal region of the primary ejaculatory duct lie closely (Fig. 7). The proximal part of the accessory gland is continuous with that of the primary ejaculatory duct where the two ducts are separated.

In 2-day pupa, the accessory glands are held together by tracheae except at the distal end. The partition between the paired primary ejaculatory ducts gradually begins to disappear at the proximal region. The membranous sheath covers the cuticle lined ejaculatory duct and now the duct consists of two tubes; inner duct and outer sheath. A constriction, not prominent appears in the middle part of the enlarged portion at the proximal end of the vas deferens and it separates the enlarged portion into proximal and distal part in 3-day pupa. The distal part becomes seminal vesicle and the proximal part continues as upper vas deferens. The vas deferens connecting the seminal vesicle with the primary ejaculatory duct remains as lower vas deferens. The partition between the paired primary ejaculatory ducts is completely disappeared at the proximal region and it becomes the primary ejaculatory duct unpaired; the remaining part continues as primary ejaculatory duct paired. Between the cuticle lined ejaculatory duct and primary ejaculatory duct unpaired, another constriction is noted, but not conspicuous.

The reproductive tract is fully differentiated in 4-day pupa (Fig. 8). The outer membranous sheath of the cuticle lined ejaculatory duct is continuous with the aedeagus through which the lumen of the cuticle lined ejaculatory duct passes. Data on table 2 indicates that the reproductive tract increases considerably during early and mid-pupal periods. The vas deferens and seminal vesicle are formed of single layered epithelial cells with distinct nucleus. The accessory reproductive glands-accessory glands and primary ejaculatory ducts both paired and unpaired consist of columnar cells, which are single layered. No secretion is noticed in the lumen of the accessory reproductive glands, but small granules or vesicles being liberating from the cells are observed in 4-day pupa. The secretion begins to appear in the accessory reproductive glands in 5-day pupa and maximum amount is noticed in 0-day adult.

Discussion

The testis of *Opisina arenosella* resembles those of other Lepidoptera in location and structure. In *Opisina arenosella* each testis is attached to the body wall by two epithelial strands as in *Porthetria dispar* (Salama, 1976) and the tracheae are seen attaching to the left and right sides of the testis as described for *Manduca sexta* (Reinecke *et al.*, 1983). The fusion of the testis in *O. arenosella* occurs in the prepupal stage as reported for *Manduca sexta* (Reinecke *et al.*, 1983), *Boarmia selenaria* (Scheepens & Wysoki, 1985) and *Spodoptera litura* (Sridevi *et al.*, 1989). In the present study the torsion of the testis takes place in 6-day pupa as a result the left and right follicles are twisted to the opposite sides respectively.

The tracheal branches from the left and right spiracles cross the testis to the opposite sides. This tracheal pattern results from the torsion of the testis. This observation is in close agreement with the torsion of the testis in *Manduca sexta* (Reinecke *et al.*, 1983). The testis enclosed within a peritoneal membrane and each follicle is covered by an outer and inner membrane in *O. arenosella*. The four follicles are covered by another membrane. This is comparable to the condition in *Porthetria dispar* (Salama, 1976).

The phase contrast studies reveal that two types of spermatid cysts-eupyrene and apyrene are distinguishable in *O. arenosella* as in *Calpodes ethlius* (Lai-fook, 1982). Though the eupyrene sperm bundles are first noticed in the late larval period, the apyrene sperm bundles appear later, only in the early pupal stage as in *Porthetria dispar* (Salama, 1976) *Papilio xuthus* (Numata and Hidaka, 1980) and *Calpodes ethlius* (Lai-fook, 1982).

The development and differentiation of male reproductive tract in *O. arenosella* conform to the pattern reported for *Manduca sexta* (Reinecke *et al.*, 1983) and *Ostrinia nubilalis* (Jones *et al.*, 1984). In *O. arenosella* the genital cord is the only reproductive tract in larva, which has no lumen and therefore it is termed as genital cord. Lumen appears in the genital cord in prepupa and now it becomes the vas deferens. A comparable condition is reported for *Ostrinia nubilalis* (Jones *et al.*, 1984). In *O. arenosella* the rudiments of accessory reproductive glands appear in the late prepupal stage. In contrast to this, in *Ostrinia nubilalis* (Jones *et al.*, 1984) they are rudimentary in last (V) instar larva since the ampullae are formed at the end of the IV instar larva. In *Manduca sexta* (Reinecke *et al.*, 1983) the glands begin to differentiate in the early stage of pupal period. The development pattern of the accessory reproductive glands in *O. arenosella* is similar to that observed in *Ostrinia nubilalis* (Jones *et al.*, 1984). The primary ejaculatory ducts are paired in *O. arenosella* (two lumina within a common sheath) as they arise and the partition between the lumen disappears in the proximal part resulting a single duct, the primary ejaculatory duct unpaired as in *Manduca sexta* (Reinecke *et al.*, 1983) and *Ostrinia nubilalis* (Jones *et al.*, 1984).

In *O. arenosella* the aedeagus and cuticle lined ejaculatory duct develop from Herold's organ in the prepupal period whereas in *Ostrinia nubilalis* (Jones *et al.*, 1984) it has been reported that cuticular ejaculatory duct and aedeagus differentiate from Herold's organ in last instar larva. However, in *Manduca sexta* (Reinecke *et al.*, 1983) the above structures emerge from the genital disc in early pupal period.

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Behavioural Responses to Humidity Gradient by *Antrocephalus hakonensis* and Other Parasitoids of *Opisina arenosella*, the Caterpillar Pest of Coconut

T. P. Mohandas & U. C. Abdurahiman
Department of Zoology, University of Calicut,
Calicut University P. O., Kerala, India 673 635

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Abstract: The behavioural responses of *Antrocephalus hakonensis* (Ashmead) to humidity were tested and compared to those of *Brachymeria nosatoi* Habu (Hymenoptera: Chalcididae), *Bracon brevicornis* Wesmael (Hymenoptera: Braconidae) and *Goniozus nephantidis* (Muesebeck) (Hymenoptera: Bethylidae). *A. hakonensis* and *B. nosatoi* preferred lower humidities than the other parasitoids. These findings have relevance to the habitat preference of the parasitoids.

Key words: Humidity responses, *Antrocephalus hakonensis, Opisina arenosella.*

INTRODUCTION

It is well known that extraneous factors like temperature and humidity have a profound influence on habitat selection in insect parasitoids, which in turn regulate their population. The importance of *Goniozus nephantidis* in the populatiбn dynamics of its host *Opisina arenosella* Walker, the black-headed caterpillar pest of coconut was noted by many authors (Antony and Kurian, 1960; George *et al.*, 1977; Nadarajan and Channa Basavanna, 1980; Dharmaraju, 1963; Manjunath, 1985); similarly, that of *Bracon brevicornis* (Ghosh and Abdurahiman, 1985) and *Brachymeria nosatoi* (Joy and Joseph, 1973, 1977, 1978; Pillai and Nair, 1981, 1982) as exerting a control over the same host were also well established. In the present study, the behavioural responses of *Antrocephalus hakonensis* (Hymenoptera: Chalcididae) on a Relative Humidity gradient was recorded and compared with that of another pupal parasitoid *Brachymeria nosatoi* and of two larval parasitoids, *Bracon brevicornis* and *Goniozus nephantidis* of *O. arenosella*. As the norms for release of *B. nosatoi* and *G. nephantidis* together with other parasitoids of *O. arenosella* have already been set (Sathiamma *et al.*, 1987), this study may provide further guidelines for the release of parasitoids in the proper habitat.

MATERIALS AND METHODS

The experimental set up (Fig. 1) was similar to that used by Weseloh (1979). Twentyfour 30 ml capacity plastic cups in 3 x 8 array were placed within a 26.5 x 10 x 4.5 cm plywood container; and 20 ml KOH solution was placed in each short row. Differing concentrations of KOH were used to give a gradient of Relative Humidities as follows: *ca* O (KOH pellets), 15,30,40,60,70,85, and 100% (distilled water) (Solomon, 1951). A piece of curtain fabric stretched over a one-cm wide plywood collar separated the insects from the solutions. A glass plate is placed above the collar to confine the parasitoids to the one cm thick space above the fabric.

Two gradient devices were used at the same time. Ten females of the same species were tested in one gradient, with a different species used in the other gradient. A scale divided into eight equal parts was placed on one side of the wooden collar just below the covering glass and the distribution of the parasitoids along the

humidity gradient was monitored with an Olympus OM-2 camera. Photographs of the parasitoids were taken at 5-min. intervals for 30 min/run (6 photographs). Each device was rotated 180° between runs to cancel out any possible extrinsic effects due to positioning of the device. The whole set up was uniformly lighted from above. After developing the film, the position of each insect was noted and associated with a particular humidity. Seven replicates were run for each species, out of which the first and seventh were run after removing all the cups to serve as controls. Each species was tested, first when provided with honey and water until just before the tests were run and then the same individuals after being deprived of honey and water for 24 hours. Gradient of humidity in each device was tested before and after the test, using filter paper impregnated with cobalt chloride.

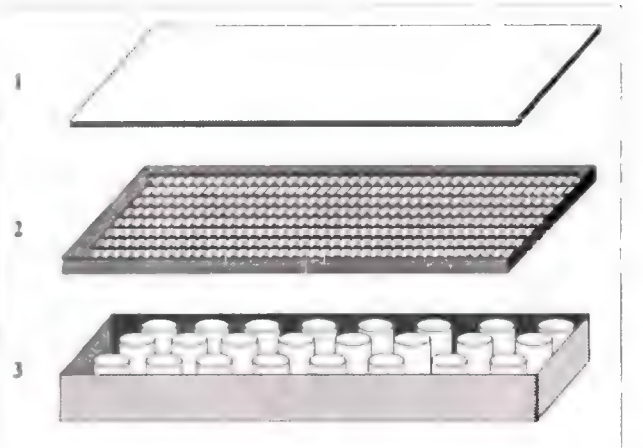

Fig. 1. 1-3. Experimental devices used to test the humidity response of the parasitoids. 1. Glass plate; 2. Fabric floor; 3. Container with cups.

All the counts were added to obtain the average number per run for each species. The data obtained for different species are graphically represented in Fig.2 The distribution of each species along the gradient was subjected to a 'goodness of fit' (Chi-square) test.

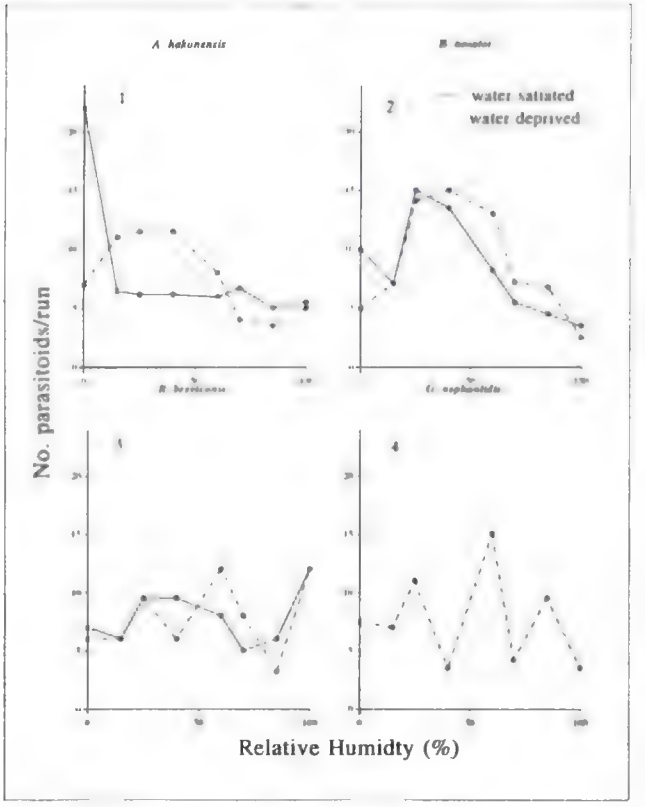

Fig. 2. Humidity response of few parasitoids of *Opsina arenosella*. 1. *Antrocephalus hakonensis*; 2. *Brachymeria nosatoi*; 3. *Bracon brevicornis*; 4. *Goniosus nephantidis*.

RESULTS

The 'goodness of fit' test showed that the distribution of insects in any of the experimental runs was not uniform except in the case of *Bracon brevicornis*. A strong preference for the dry end of the humidity gradient was shown by water-satiated females of *A. hakonensis* (X^2= 27.69, P<0.01) and *Brachymeria nosatoi* X^2 = 18.93, P<0.01). The same individuals after 24 hours of water-deprivation still showed marked preference for the dry zone, with *B. nosatoi* showing the same pattern of distribution (Fig.2.2) as shown by the water-satiated individu-

als (X^2= 20.26, P<0.01) and *A. hakonensis* slightly avoiding the driest zone (Fig.2.1) (X^2= 15.47, P<0.05). Both water-satiated and water-deprived females of *B. brevicornis* showed a more or less uniform distribution along the gradient (Fig.2.3). However, water deprived (only one set tested) *G. nephantidis* were irregularly distributed (Fig.2.4) along the gradient with higher number on the humid half of the gradient (X^2= 16.53, P<0.05). Distribution of insects in all control tests were uniform except in one test where water-satiated *B. nosatoi* females unusually congregated in the middle of the gradient.

DISCUSSION

Experimental results indicate preference for lower humidities by *Antrocephalus hakonensis* and *Brachymeria nosatoi* when compared to *Bracon brevicornis* and *Goniozus nephantidis*. Humidity preference by the two chalcid species agree quite well with earlier results obtained for some *Brachymeria* spp. For example, *B. intermedia* selected warm bright and dry sites on gradient (Minot and Leonard, 1976). *B. intermedia, B. lasus* and *Brachymeria* sp. preferred lower humidities and higher temperatures than other common gypsy moth parasitoids and hyperparasitoids, thus they may occupy microhabitats different from others (Weseloh, 1979). These observations are also supported by earlier field studies which showed that *B. intermedia* prefer open, sunny areas where temperatures are high and humidities low (Doane, 1971; Leonard, 1971 and Weseloh, 1972).

Opisina arenosella is a widely distributed pest and occupies habitats ranging from coastal and brackish water areas to inlands (Rao *et al.*, 1948; Dharmaraju, 1952; Nirula, 1956; Tirumala Rao and Mohan Rao, 1953; Joy and Joseph, 1972; Sathiamma *et al.*, 1973; Nadarajan and Channa Basavanna, 1980; Perera, 1988). The distribution and seasonal occurrence of the parasitoids were also shown to vary considerably. *Goniozus nephantidis* is generally active throughout the year with higher incidence in February and March and lower incidence during April and June in Bangalore (inland area) (Nadarajan and Channa Basavanna, 1980). The same authors observed that *Brachymeria* spp. were adversely affected by the high humidities in September and consequent increase in host pupal population in October. The greater activity levels of *G. nephantidis* at 70-80% Relative Humidity (Dharmaraju and Pradhan, 1976; Nadrajan and Channa Basavanna, 1978) and *Bracon brevicornis* at 70% R. H. (Sharma, 1956) more or less correspond with their humidity preference found in the present study.

Opisina arenosella is most active during the hot and dry months of the year, chiefly from March to May in South India (Nirula, 1956; Narayanan, 1954; Joy and Joseph, 1972); therefore, the need for the presence of the population of those parasitoids that can grow vigorously under hot climate, such as *B. nosatoi*, which has peak activity in May, has been suggested to be inevitable for effective control of the pest (Joy and Joseph, 1978).

In conclusion, it is presumed that *Antrocephalus hakonensis* and other chalcidid parasitoids can be more effective when compared to the larval parasitoids, in checking the population of *Opisina arenosella* in the open dry areas during the peak period of summer.

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Four New Species of Histiostoma Associated With Insects in Tamil Nadu

C. Chinniah and M. Mohanasundaram
Department of Agricultural Entomology, Tamil Nadu Agricultural University
Coimbatore - 641 003, India

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Abstract: The paper presents the discriptions and figures of four new Anoetid mites phoretic on insects in Tamil Nadu viz., *Histiostoma coprae* sp. nov. on *Coprus* sp (Coprinae: Scarabaeidae. Coleoptera): *H. muscae* sp. nov. on *Musca domestica* L. (Muscidae: Diptera): *H. onthophagae* sp. nov. on *Onthophagas pactolus* F. (Scarabaeidae: Coleoptera) and *H. siphunculinae* sp. nov. on *Siphunculina sp.* (Chloropidae: Diptera)

Key words: Histiostoma: Anoetidae, Phoretic mites.

INTRODUCTION

In the course of screening and study of mites associated with insects in Tamil Nadu, several species of mites new to science were encountered. The paper presents the descriptions and figures of four species of *Histiostoma* (Anoetidae: Acari) collected from insects. From India only one species *H. prophthalmi* (Vitzthum) has been recorded from Assam so far (Prasad 1974). The Anoetidae are usually found in damp places such as in the sap of trees around wounds, in rotten damp fungi, in *Drosophila* cultures, in rotting potatoes, and in other similar habitats. The duetonymphs or hypopial forms are found on insects (Baker and Wharton 1952). In this investigation also the phoretic stages have been isolated from the insect hosts. The type and paratype slides have been deposited in the Acarology collections of the Department of Agricultural Entomology, TNAU, Coimbatore 641 003, India. In the descriptions all measurements are given in microns.

Histiostoma coprae sp. nov (Figs 1 to 4).

Hypopus: Hypopi with a body length of 174 and a width of 146. Body oval shaped, setation reduced and dorsum with a few number of setae, propodosomal shield distinct with two pairs of small setae 4 long. Hysterosoma with 8 pairs of small setae; caudal setae 5 long at the posterior of dorsal shield; dorsum without any distinct patterns and punctations, completely covering the gnathosoma.

Venter - In ventral side also the chaetotaxy is very much reduced but the setae are comparatively longer than the dorsal setae, 7 long, only four pairs of setae are distinctly seen. The aopdemes of leg I to IV are free. The ventral sucker plate is as figured, and distinctly different from the previously known species. Gnathosoma is reduced and represented by a pair of comparatively short seta, 18 long, the subcapitulum 8 long and the palp 6 long.

Legs - Leg characters typical to the genus *Histiostoma*. However the coxae of legs I and II bears a pair of prominant spur like process 4 long one on the dorsal side and the other on the ventral side. Legs III and IV are slender than the other two pairs and directed forward. Leg I, 104 long, had a sensory rod 16 long on the tarsus and an empodial sucker 30 long w which is lanceolate and quite characteristic in this species, but the empodial claws are absent in all the legs. Leg II, 84 long, bears a sensory rod almost equal to the length of tarsus, 10 long and ends in a lanceolate empodial sucker 20 long as in the 1st pair of legs.

Leg III, 50 long, also bears the empodial sucker 16 long but devoid of the sensory rod.

Leg IV, 60 long without either an empodial sucker or sensory rod but ends with a long prominant simple seta 24 long. All the segments of the legs I to IV excepting the coxae bears simple setae common to the Anoetid

mites.

Types: One holotype hypopus marked on slide India: Tamil Nadu: Coimbatore: 10. V. 1988 ex. *Coprus* sp. (Corprinae: Coleoptera). Coll. C. Chinniah: (No. 36/2). Two paratype slides with 5 hopopi on each slide. Collection data same as the holotype.

Diagnosis: This new species resembles *histiostoma pulchrum* Kramer (Hughes 1976) in the general facies and shape but differs from that in the shape of apodomes of legs I to IV, the structure of ventral sucker plate, the presence of spurs on the coxae of all the legs, presence of lanceolate empodial discs instead of the empodial claw in the legs I to III.

Relationship to the host: These straw yellow coloured mites were isolated from the beetles collected from light traps, glued to the ventro-lateral sides of the abdomen, just below the wing base as well as to the ventrum of thorax. All the mites isolated were hypopi which is a specially adopted form for phoresy among anoetids with reduced gnathosomal structures and the well developed ventral sucker plate meant for attachment and hence it is purely phoretic on the beetle hosts.

***Histiostoma muscae* sp. nov.** (Figs 5-7)

Hypopus: The mite has an oval body measuring 162 long by 124 wide minute punctuation all over the dorsum. The propodosomal shield is not as distinct as in the previous species. Just below the anterior margin of hysterosoma fine wavy patterns are seen. Gnathosoma is reduced and represented by the long Omega (w) setae 52 long and a subcapitulum 20 long and 6 wide.

Venter: The apodemes of legs I and II on the ventral side are prominant and open but the apodemes of the legs III and IV are closed. The ventrum is completely devoid of setation. The suctorial plate is as figured with lesser number of discs, the outer disc anterior to the inner disc. The suctorial disc is quite different from the other known species.

Legs: Legs are weekly sclerotized, legs III and IV are slender, anteriorly projecting, ending in a long whip like setae as usually found in Anoetids. Leg I about 104 long and all setae are setaceous excepting the one arising from the tibia which is very long and whip-like 70 long. It also has a sensory rod (Seta) 18 long almost equal to half the length of tarsus. The tarsus ends in an empodial claw 7 long and a empodial rod 12 long. Leg II, 88 long, has the usual characters of anoetids but with an empodial rod 13 long and claw 7 long. Leg III, 80 long with whip like setae 24 long on genu similar to the one with which the tarsi ends. The legs III and IV have long tibia and tarsus without a prominent empodial claw. Leg IV measures 30 long with a long terminal seta 42 long.

Types: One holotype hypopus marked on slide India: Tamil Nadu: Coimbatore: 7. VI. 1988. ex. *Musca domestica* L. (Muscidae: Diptera): Coll. C. Chinniah (No.43/1) Three paratype slides each with 4 hypopi. Collection data same as holotype.

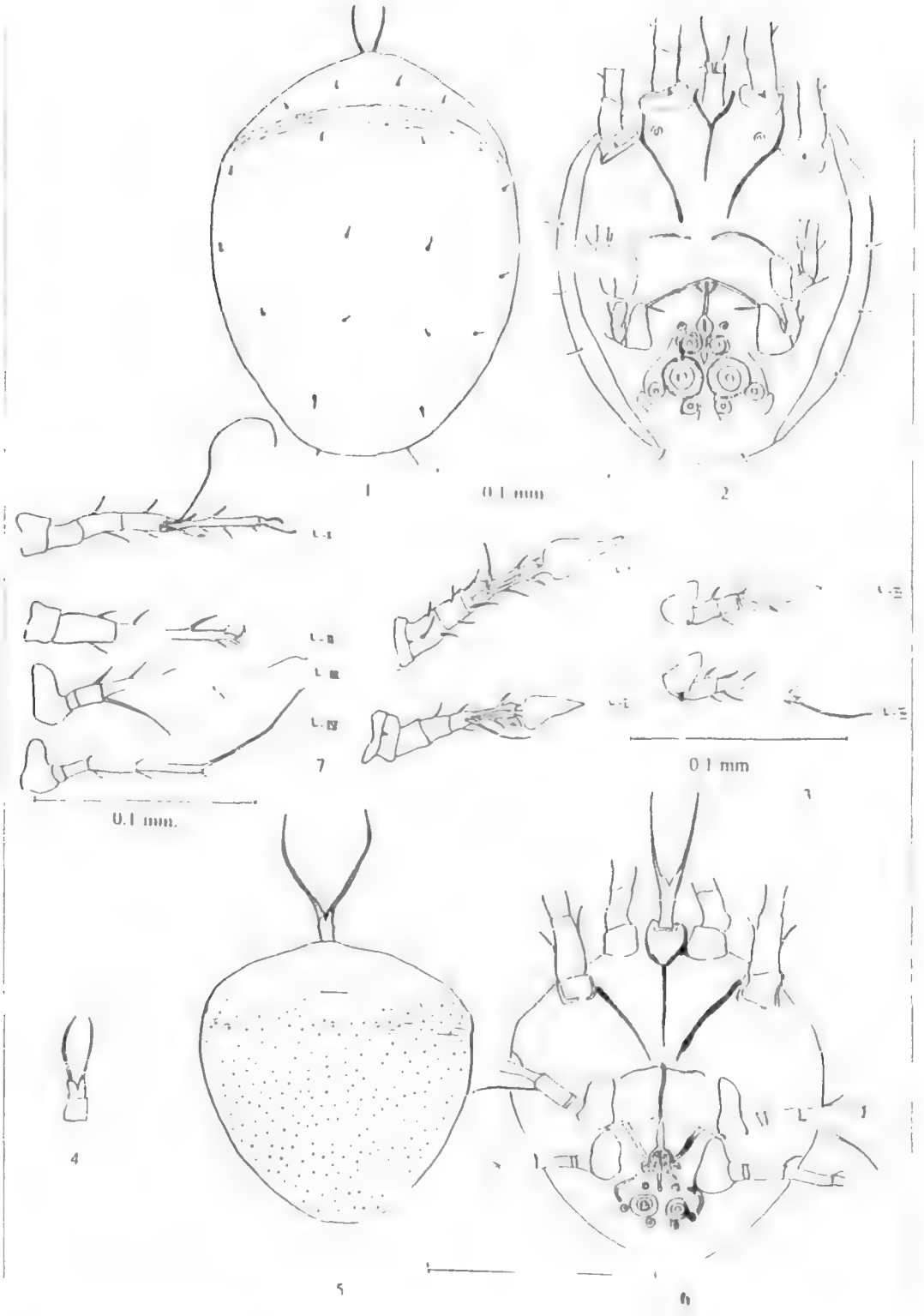
Diagnosis: The new species described here resembles *Histiostoma discrepans* Oudemans (Baker and Wharton 1952) in general structure, leg chaetotaxy and dorsal body poroidotaxy but differs in the structure of the apodemes and the disc arrangement in the sucker plates.

Other materials studied - The same species of mite has been isolatd from the stable flies *Stomoxys calcitrans* (Muscidae: Diptera) collected from meat shop for the observation of other parasitic trombidids. These hypopi were found attached just below the wing base, sides of the abdomen and also on the venter of abdomen and thorax.

Relationship to the host: Hypopi (light yellow coloured) were isolated from the common hourse flies collected for the observation of other parasitic trombidids. Several hypopi were found to be firmly attached to the wing bases and other parts of the body amidst the long body hairs. In fact, all were hypopi with characteristic adaptations and hence it would be a specialised form of phoresy.

***Histiostoma onthophagae* sp. nov.** (Figures 8 to 11)

Hypopus: Oval shaped body, the dorsum measuring about 194 long by 144 broad. The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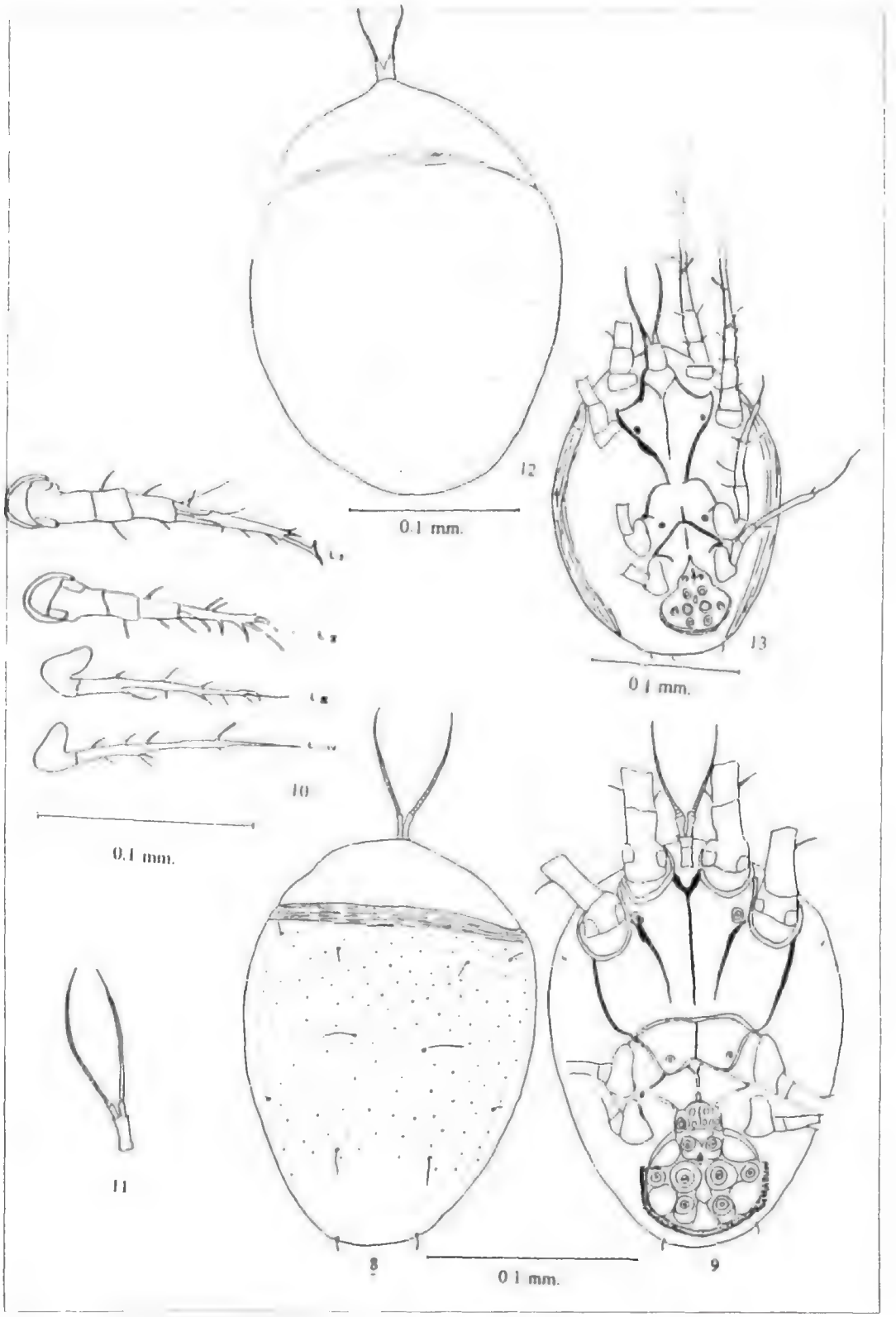
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hysterosoma with many fine pores but without any specific pattern. The propodosoma without any setae but the hysterosoma bears six pairs of thin setae, setation is however very sparce, a pair of setae present at the extreme posterior of the hysterosoma. The anterior of hysterosoma has a light pattern of wavy lines. Gnathosoma despite reduced 24 long and 8 wide, well represented by a pair of setae 40 long extending past the anterior margin of propodosoma.

Ventrum: The apodemes on the ventrum are very prominant and characteristic. The ventrum measures 200 long, 146 wide, sternum almost reaching the posterior margin of sternal plate. The sternum completely devoid of any setation. The apodomes I and II open, III and IV enclosed. The suctorial plate as figured, measuring 46 long and 60 wide. The outer disc placed anterior to inner disc.

Legs: Legs I to IV weekly sclerotized, coxae without any setae, other segments bear long, thin setae. The tarsi I ends with empodial claws and empodial rods: tarsi II also bears a long lanceolate seta and empodial claw 6 long: tarsi IV simply ends in a long whip like setae 32 long. The length of legs I to IV 140, 110, 84 and 120 respectively. Legs III and IV are more slender than I and II, and the tibia and tarsi of legs III and IV are more slender and longer than in other legs.

Types: One holotype hypopus marked on the slide, India: Tamilnadu: Coimbatore: 12. V. 1988 ex *Onthophagus pactolus* (F) (Scarabaeidae: Coleoptera). Coll. Chinniah, (No: 37/2). Three paratype slides each with 3 hypopi collection data same as the type.

Diagnosis: This new species resembles *Histiostoma sapromyzarum* Dufour (Huges, 1976) in general facies and leg characters but clearly distinct in the structure of ventral apodeme and the sucker plate. Other characters are typical to the genus, *Histiostoma*.

Relationship to the host: The light yellow coloured hypopi isolated from the scarabaeid beetle collected from light trap. These hypopi observed to be firmly attached to the sides of the ventrum of abdomen and thorax since all the hypopi with special adaptation for phoresy such as reduced gnathosoma, sucker plate, etc., the relationship proved to be a specialized phoresy.

***Histiostoma siphunculinae* sp. nov.** (Figures 12 to 15)

Adult female: Body 550 long and 200 wide. The dorsal setae are comparatively long and smooth. The propodosoma bears a pair of setae Sci and Sce; Sci is shorter 18 long than the Sce, 80 long, setae is also shorter in length equal to laterals. Setae d2 is longer than all other dorsal setae like d3 and d4. A narrow groove separates the propodosoma from rest of the body dorsally.

Venter: On the ventral surface, the notable feature is the two pairs of chitinous rings which are oblong. The anterior pair lying between coxae II and III on either side of the transverse genital opening, the posterior pair lying just below coxae IV. According to Perron (1954) these may function as osmoregulating organs, alternatively they could indicate the position of muscle insertions. The apodemes of leg I just meet in the midline, those of legs II to IV are shorter, and their internal ends widely separated. The ventral setae are small and limited in number, two pairs of anal setae are present.

Gnathosoma: It is rather small in proportion to the rest of the body 120 long, the chelicerae have only one saw like chela and the palpal tarsus has two conspicuous, laterally projecting structures.

Legs: The legs are short and think ending in a stout claw, the setae are thickened to form spines, one (ba) lying immediately in front of Omega I. On tarsi I and II, Omega I arises from the extreme base of tarsus I. On tarsus II Omega I occupies its normal position and is only slightly curved.

Hypopus: Oval shaped body and dorsum is fully covered by the dorsal carapace, propodosomal shield is distinct. The apodeme structure as figured and the sucker plate has more than 4 pairs of discs. Ventral setation is mich reduced. Gnathosoma reduced and represented by sub-capitulum 15 long and seta omega 50

long.

Legs I and II have Omega 1 and Omega 2, 12 and 10 long respectively, tarsi I has a modified setae at the tarsal tip. Legs III and IV, 100 and 110 long, have long slender tibiae and tarsi ending in long setae.

Diagnosis: This species resembles *Histiostoma feroniarum* Dufour (Hughes 1976) in ventral adult characters as well as the characters of hypopus; but the adult dorsal chactotaxy varies and setal length also is more. In the hypopus the apodeme structure varies from *H. feroniarum*. Leg chaetotaxy also varies distinctly.

Types: A holotype female marked on the slide India: Tamil Nadu: Coimbatore; 15. VII. 1988 ex. Chloropid fly *Siphunculina* sp. (Chloropidae: Diptera) Coll. C. Chinniah (No (29/1) One paratype slide (29/2) with 4 females, and two more paratype slides (29/4 &3) each with 5 hypopi. Collection detail same as type.

Relationship to the host: Hypopus isolated from the chloropid flies collected for the routine examination of other ectoparasitic mites. These phoriants found to occur just below the wing bases and ventrum of thorax. The adults and the hypopi of the same species were collected from the rotten sorghum shoot that would have been attacked by sorghum shoot fly previously. From the observation it is likely that these chloropid flies (Carrier) would have oviposited in the rotten shoot and when the adult flies emerges out these mites (hypopus) have turned to be phoretic. So the relationship is a specialised form of phoresy.

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[Brief Communication]

Leaf Folder Resurgence - A Side Effect of Insecticide Application in Rice Field

S. Devanesan, Vijayaraghavakumar, V. Ramachandran Nair, Thomas Biju Mathew, S. Ravi and A. Visalakshi

Kerala Agricultural University, Cropping Systems Research Centre
Karamana, Thiruvananthapuram, Kerala - 695002

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The paper reports the results of screening of five granular insecticides, for their resurgence inducing property to Rice Leaf folder, *Cnaphalocrocis medinalis* Guenee. Carbofuran was the only insecticide identified as causing resurgence to *C. medinalis* irrespective of the dosage applied while cartap, diazinon, quinalphos and phorate controlled the pest.

Key words: granular insecticides, rice leaf folder, resurgence.

Systemic granular insecticides like carbofuran, phorate etc. are widely used for paddy pest control especially in rice growing tracts of Kerala where plant and leafhoppers, gall midge, stem borer and other pests are regularly occurring. In spite of the effective control of these target pests, severe infestation by *Cnaphalocrocis medinalis* was frequently observed in carbofuran treated plots when compared with untreated plots. This unusual phenomenon observed as a side effect of insecticide application in rice was studied in detail at the Cropping Systems Research Centre, Karamana (Thiruvananthapuram) and the results are presented in this paper.

In the first experiment conducted during the Virippu season 1989, five granular insecticides viz. cartap, diazinon, quinalphos, phorate and carbofuran were screened for resurgence inducement to *C. medinalis*. Insecticide formulations (Vide Table I) were procured directly from the manufacturers. Annapoorna, a susceptible variety was used for the experiment. First treatment was given in the nursery beds on 10th day after sowing (DS). Fourth leaf stage seedlings were transplanted in 5 x 4 m^2 plots at a spacing of 15 x 10 cm. Completely Randomised Block Design (CRBD) was adopted for the experiment and each treatment was replicated three times. In the main field treatments were given twice at 20 and 40 days after transplanting (DT) and all the insecticides were applied at 1 Kg ai/ha in the nursery as well as in the main field. Extent of damage by *C. medinalis* was assessed twice at 7 and 14 days after the last treatment adopting standard techniques (Heinrichs *et al.*, 1981). This experiment was repeated in Mundakan, 1989 also.

The second experiment was conducted during Virippu season of 1990 to study the influence of dosage of carbofuran on the degree of resurgence of *C. medinalis*. Carbofuran was applied thrice at two doses 1 and 2 Kg ai/ha as done in the first experiment. The experiment was conducted in a CBRD and treatments were replicated eight times. Intensity of damage by *C. medinalis* was assessed at 7, 14 and 28 days after the last application. Since the control plots showed damage by rice stem borer *Scirpophaga incertulas* the percent white earheads in treated and control plots were also recorded.

Results of the two trials conducted during Virippu and Mundakan seasons of 1989 are presented in Table 1.

At one week after the last treatment (47 DT) the extent of damage caused by *C. medinalis* was significantly less in plots treated with diazinon, quinalphos and phorate when

Table 1. Effect of different granular insecticides on the incidence of rice leaf folder, ***Cnaphalocrocis mendinalis***.

Insecticide	Formulation	Mean per cent leaves damaged by *C. medinalis* observed at					
		Virippu 1989			Mundakan 1989		
		Before treatment (40 DT)	One week after (47 DT)	Two weeks after (54 DT)	Before treatment (40 DT)	One week after (47 DT)	Two weeks after (54 DT)
Cartap	Padan 4g	12.37 (20.58)	20.6 (26.98)	27.25 (20.97)	7.70 (16.11)	15.28 (23.0)	14.61 (22.47)
Diazinon	Basudin 10G	11.51 (19.82)	18.51 (25.46)	15.52 (18.57)	7.28 (15.65)	13.49 (21.54)	13.49 (21.54)
Quinalphos	Ekalux 5G	14.87 (22.67)	16.41 (23.88)	26.05 (19.28)	9.39 (17.84)	11.21 (19.55)	14.70 (22.54)
Phorate	Thimet 10G	13.85 (21.84)	19.50 (26.20)	27.11 (20.76)	8.88 (17.34)	14.53 (22.40)	15.38 (23.08)
Carbofuran	Furadan 3G	13.19 (21.29)	46.41 (42.92)	41.09 (43.21)	8.39 (16.84)	35.54 (36.58)	33.79 (35.53)
Control		15.23 (22.96)	27.82 (31.82)	35.45 (33.68)	11.71 (20.01)	15.89 (23.48)	22.25 (28.27)
CD (0.05)		(NS)	(5.29)	(6.96)	NS	(4.24)	(3.53)

1. All insecticides were applied at 1 kg ai/ha once in the nursery (10 DS) and twice in the main field (20 & 40 DT). 2. Figures in parentheses are transformed values, angles.

compared with that in control plot while in cartap, the damage came on par with that of control. In plots treated with carbofuran, the damage by *C. medinalis* was significantly higher than in control plots.

At 2 weeks after the last treatment the intensity of damage caused by *C. medinalis* in plots treated with cartap, diazinon, quinalphos and phorate was significantly less than in the untreated plots while the damage observed in carbofuran treated plots was the highest.

When the experiment was repeated with the same set of treatments during Mundakan 1989 almost a similar trend was observed at 2 weeks after treatment. Cartap, diazinon, quinalphos and phorate controlled the damage while carbofuran induced significant resurgence of *C. medinalis*.

As resurgence inducing property of carbofuran was consistently observed in the first experiment, the influence of dosage on the degree of resurgence was assessed in the second experiment and the data relating to this are presented in Table 2. The mean per cent damage caused by *C. medinalis* in plots treated with carbofuran at both the doses did not differ significantly as observed at 7, 14 or 28 days after the last treatment. At all the three intervals significantly higher damage was observed in treated plots when compared with control plots and the degree of resurgence was independent of the dosage tried.

Mean percent white earheads caused by *S. incertulas* observed at harvest, indicated that carbofuran significantly reduced the damage at both doses tested.

Consistent resurgence of rice leaf folder following application of carbofuran significantly reduced the damage at both doses tested.

Table 1. Effect of carbofuran applied at two doses, on the damage caused by ***C. medinalis*** and ***S. incertulas*** on rice.

Treatments		Mean percent leaves damaged by *C. medinalis* observed at different intervals after the last treatment (days).			Mean percent white earheads observed at harvest
		7	14	28	40 DT
Carbofuran	1 kg ai/ha	17.46 (4.18)	11.35 (3.37)	11.88 (3.45)	9.56 (3.09)
	2 kg ai/ha	18.78 (4.33)	12.45 (3.53)	13.48 (3.67)	9.61(3.10)
Control		10.33 (3.21)	6.29 (2.51)	8.77 (2.96)	15.92 (3.99)
CD (0.05)		(0.51)	(0.48)	(0.36)	11.52 (0.74)

Carbofuran was applied once in the nursery (10 DS) and twice in the main field (20 & 40 DT) Figures in parentheses are tranformed values, square roots.

Consistent resurgence of rice leaf folder following application of carbofuran was evident in all the field experiments in this study. Earlier studies also indicated that carbofuran induced resurgence to *C. medinalis* (Nadarajan and Skaria 1988; Panda and Shi 1989). However diazinon an insecticide identified as resurgence inducing to brown plant hopper (Chelliah, 1979) and phorate identified to induce resurgence of *C. medinalis* (Chelliah and Heinrichs, 1984) failed to show resurgence in the present study.

In the light of the experimental evidences obtained so far on the resurgence inducing property of carbofuran, it may be concluded that while selecting an insecticide for chemical control of the pest complex in rice, the chances of resurgence inducement to a non-target species should also be considered. Detailed biochemical studies on the relation between leaf folder resurgence and phytotonic effects of carbofuran may explain the mechanism of resurgence inducement.

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[Brief Communication]

Insecticidal Activity of *Uvaria narum* Wall and *Uvaria hookeri* King against *Cylas formicarius* Fab.

V. Padmaja[1], P. Rajamma[2], V. Thankamani[3] and A. Hisham[3*]

[1]College of Pharmaceutical Sciences, Medical College, Trivandrum, Kerala, India 695 011
[2]Central Tuber Crops Research Institute, Sreekariyam, Trivandrum, Kerala, India 695 017.
[3]Department of Chemistry and Environmental Microbiology, College of Engineering, Trivandrum, India 695 016.

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Abstract: Root barks of *Uvaria narum* Wall and *U. hookeri* King were investigated for their insecticidal activity against *Cylas formicarius* Fab. Ethyl acetate extracts of both the plants show 10 to 60% mortality at 0.125 to 1.000% concentrations while hexane extracts showed only 10 to 35% mortality at the same concentrations. The acetogenins isolated from these extracts showed 10 to 50% mortality at 0.0005 to 0.002% concentrations.

Key words: Insecticidal activity, *Uvaria narum*, *Uvaria hookeri*, *Cylas formicarius*.

Recent phytochemical studies on certain species of the Annonaceae in the genera such as *Annona, Rollinia, Uvaria, Goniothalamus* and *Asimina* have resulted in the isolation of several long chain tetrahydrofuran containing compounds which are now referred to as Annonaceous acetogenins. This novel group of natural products were found to possess a broad range of biocidal activities including interesting pesticidal activities. (Rupprecht *et al.*, 1990; Zin Ping Pang *et al.*, 1993).

Our recent investigations on the root bark of *Uvaria narum* and *U. hookeri* have resulted in the isolation of several acetogenins and terpenoids (Hisham *et al.*, 1990, 1991 a and b; 1992 and 1993). As a part of our ongoing programme for evaluating the Annonaceous acetogenins as bio-degradable pesticides, we have studied the insecticidal activities of the root bark extracts and the isolated acetogenins of *U. narum* and *U. hookeri* against sweet potato weevil *Cylas formicarius* (Coleoptera: Cureulionidae) and the results are presented here.

Plant materials: Root barks of *Uvaria narum* and *U. hookeri* were collected from Trivandrum and Changanassery areas of Kerala during 1992. Known quantities of root bark of the two plants were individually extracted first with hexane and subsequently with ethyl acetate. Crude extracts were obtained after complete removal of the solvents with vacuum evaporation. All the four extracts were separately subjected to silica gel column chromatography. The acetogenins uvariamicins I, II and III, isodesacetyluvaricin and squamocin 28-one were isolated from the hexane extracts and the acetogenins narumicins I and II (stereo mixture) and squamocin were isolated from the ethyl acetate extracts.

Evaluation of insecticidal activity: 0.4 ml of different concentrations of the acetogenins in ethanol were poured into clean, glass tubes of 50 ml capacity and a uniform fill of the product was made by rolling the vials. Ten numbers of one week old adults were released into each of the treated vials which were covered with muslin cloth to prevent escape of the insects. Control vials were treated with solvent (ethanol) alone.

*Corresponding author

Table 1. Mortality of adults of *C. formicarius* at different time intervals after exposure to extracts of *U. narum* and *U. hookeri*.

Plant and extract	Concentration(%)	Mortality (%)			
		Day 1	Day 3	Day 5	Day 7
Uvaria narum Hexane	0.125	0	10	10	10
	0.250	0	20	20	20
	0.500	10	20	25	30
	0.750	20	25	25	30
	1.000	20	30	30	20
U. narum Ethyl acetate	0.125	0	10	10	30
	0.250	0	30	30	40
	0.500	10	30	35	50
	0.750	20	35	45	60
	1.000	20	40	50	10
Uvaria hookeri Hexane	0.125	0	10	10	10
	0.250	0	10	20	20
	0.500	10	20	20	20
	0.750	10	30	30	30
	1.000	10	30	35	35
U. hookers Ethyl acetate	0.125	0	10	10	10
	0.250	0	10	20	25
	0.500	10	25	30	30
	0.750	20	30	35	40
	1.000	20	35	50	60

Table 2. Mortality of adults of *C. formicarius* at different time intervals after exposure to Acetogenins.

Compound	Concentration(%)	Mortality (%)			
		Day 1	Day 3	Day 5	Day 7
Uvariamicns I, II, & III	0.0005	0	10	10	10
	0.0010	0	10	20	20
	0.0020	0	10	20	25
Isodesacetyluvaricin	0.0005	0	10	10	20
	0.0010	0	10	20	30
	0.0020	0	10	30	50
Squamocin 28-one	0.0005	0	10	20	20
	0.0010	0	10	20	20
	0.0020	0	10	20	30
Narumicins I & II	0.0005	0	10	10	10
	0.0010	0	20	20	30
	0.0002	0	20	30	40

Insects were transferred to fresh, clean tubes after an exposure period of 48 hours. Fresh sweet potato chips were provided to the adult insects from 24 hrs after the treatment. Observations on mortality were made at 1,3,5 and 7 days after the treatment. Three replication of

each treatment were used and the experiment was conducted at room temperature (28±3° C).

All the concentrations of crude extracts caused 10 to 60% morality with ethyl acetate and 10 to 35% with hexane respectively within 7 days after the treatment of adult beetles (Table 1). During this period, there was no mortality of the insects in solvent treated controls. Acetogenins caused 10 to 50% mortality within 7 days after the treatment (Table 2). Among the acetogenins, squamocin 28-one and squamocin were more active than others giving 20 to 50% and 10 to 40% mortality respectively. The present results strengthen the view that several acetogenins have selective activity and a mixture of these compounds as found in crude extracts have shown useful additive and synergistic effects.

Several plant products have been recently reported to possess insecticidal, antifeedant and repellant activities (Deshpande *et al.*, 1988; Jha & Roychowdhary, 1988 & Saxena *et al.*, 1992). Plant products such as *Calophyllum* cake, Madhuca cake (*Madhuca indica*), Lemon grass (*Cymbopogon flexuosus*) leaves of *Clerodendron infortunatum* and *Eupatorium odoratum* were reported to have insecticidal/ deterrent activity against *Cylas formicarius* (Johnson *et al.*, 1979: Rajamma, 1982). Two new plants *U. narum* and *U. hookeri* have been added here in the array of plants showing insecticidal property. In contrast to synthetic insecticides, plant products are considered to be safer and environmentally acceptable.

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